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BOTANICAL SOCIETY OF AMERICA

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VOLUME VII—1920

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ERRATA, VOLUME VII

Page 139, line two in legend, read Fig. 67 for Fig. 87.
Page 148, line 29, read plant for plane.

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THE INJECTION OF CHEMICALS INTO CHESTNUT TREES[1]

CAROLINE RUMBOLD

The rapid spread of the chestnut bark disease caused by *Endothia parasitica* (Murr.) A. and A. in the eastern part of the United States during the past ten years and the resultant appeals for help from owners of ornamental chestnut trees and of chestnut orchards, reluctant to lose their trees, were the reasons for this experimentation.

As a rule the fungous diseases of plants are such that the application of sprays, crop rotation, fertilizers, and sanitary methods of cultivation prevent or hold them in check. This disease, however, like many others to which trees especially are subject, can not be treated in this way. The cause of the sickness is in a part which can not be reached by any outside application of medicine, fertilization of the soil will not help, nor will sanitation prevent, at least in many parts of the eastern states.

The customary method of keeping such a disease in check has been to cut away and burn the diseased parts or the entire trees. The money value of the individual trees caused discontent with this method.

Experiments on tree injection were undertaken as a possible remedy. It was believed that from its nature this treatment could not be applied to forest trees. Only such trees as had a definite commercial or aesthetic individual value would repay the requisite cost and trouble.

HISTORY OF PLANT INJECTIONS

The idea of introducing foreign substances into plants is two centuries old. In 1709 Magnol (cited by Sachs, 1) introduced colored solutions into plants in order to find through what channels the sap passed. These first injections were made by placing the cut stems of leaves, twigs, or flowers in the solutions. McNab (2) was the first to put lithium into trees. He used caesium as well. About this time Pfitzer (3) injected salts of thallium. In 1887, Gaunersdorfer (4) published the results of seven years of experimentation on the effect of lithium sulphate on plants. He injected small conifers without injuring them and found that the plants finally eliminated the salt introduced into them through the roots by throwing it off with the leaves and bark. He believed young shoots, leaves, and reproductive organs were protected from the lithium by the lack of lignified water-transferring tissue. Physiologists such as Sachs (5), Strasburger (6), Wieler (7), and Pfeffer (8) established the fact that some substances foreign to plant tissues could be safely conducted through them. At the same time a large number of substances were found to be poisonous. In general, the response given by the plants to the poisons resembled that given by animals, *i.e.*, a very small amount of poison could be introduced into them without injury or noticeable change; a still larger amount increased their activities, often their growth; a larger amount retarded their activities, while still more killed. A plant could furthermore become accustomed to a poison to a certain limit, provided the poison was introduced into it in small quantities at first and these doses gradually were increased. Doses could in the end be administered without detriment that would otherwise have killed at once.

---

[1] The Pennsylvania Chestnut Tree Blight Commission was responsible for the starting of this experimental work in 1912; Investigations in Forest Pathology, Bureau of Plant Industry, for its continuation in 1913–1914 and in part for its continuation until 1918. The University of Pennsylvania furnished laboratory facilities, greenhouse, and many supplies. To Mr. Harold Peirce of Philadelphia, Secretary of the Commission, belongs the credit that these experiments were continued to their present stage.

The idea of injecting trees for purposes of wood preservation is also old. In 1840 and 1841, Boucherie (9) published accounts of experiments in which chemicals were injected into living trees. His method of injection killed the tree. The introduced liquid was distributed up and down the trunk, the injected area decreasing rapidly in breadth toward the roots. Fall was the best season for a complete saturation by this method, but it could be done in the spring. Coniferous trees were an exception because sap movement took place in them throughout the winter. Different substances were absorbed at different rates; neutral salts penetrated the wood in large quantities, acids and alkalies to a less extent. If there were hard knots or rotten spots at the base of the tree, the whole strip of wood above them would not be saturated at all. The same was true of the old wood of hard wood plants. Boucherie's ideas were used by Shevyrev in his work.

The first paper on tree injection for purposes of medication was that by Ivan Shezyrez (J. Shevyrev, Schewirew or Chewyreuv) (10). The most of Shevyrev's experiments on the injection of living trees were made with stains for the purpose of establishing the fact that solutions of substances foreign to tree tissues could safely be introduced into trees. He mentions injecting grape vines with copper sulphate but does not give the results. He describes his methods of injection and his theories as to tree injection as follows:

The best time for injection is the late summer and fall. The liquid is distributed to all parts of the tree, with the exception of the dead portions. The liquid enters the roots as well as the leaves, twigs, and fruits. This current takes the place of the sap, ascending and descending, the only difference being that it is an unusual (extraradicate) instead of the usual (radicate) current. The duration of this created second sap movement does not exceed five days. The most intensive absorption takes place at the beginning, gradually diminishes, and ceases entirely in from three to five days. He believed this diminution and cessation due to the obstruction of the vessels. Shevyrev found that the weather greatly influenced the rate of intake; he made a record of the hourly intake of an injected grape vine and of the weather for a period of three days, which showed that the consumption at night was less in quantity than that in the day, regardless of the weather.

Shevyrev's experiments were made primarily for the purpose of destroying such insects as injure plants by burrowing beneath the bark. He believed, however, that fungous diseases could be cured by the same method.

Shevyrev did not continue his experiments. The last paper (11) he published on the subject describes and criticizes the injection experiments of some Russian workers who had been treating diseased trees. He speaks of the experiments of K. K. Reshkv or K. Reschko in the Crimea, to which no other reference could be found by the present writer. Reschko treated in 1901, according to Shevyrev, a thousand trees suffering from chlorosis by introducing iron sulphate into canals cut in the bases of the diseased trees. The distribution of the substance was found to be irregular, so that individual branches were found to be uninjected.

Pachassky (12), in a governmental report of 1903, reported favorably on the injection of iron sulphate either in powder or in solution in the treatment of diseased fruit trees.

C. A. Mokrjetsky (S. A. Mokrzecki or Mokrzhetski) (13), in governmental reports of 1902 and 1903, tells of injecting more than 500 trees, the method of injection being analogous to Shevyrev's. Diseased apple trees were cured with iron sulphate, gummosis of apple, pear, and other trees with 1 percent salicylic acid. He injected "nutrient solutions" into frost bitten trees, which recovered rapidly after treatment and grew three times as much as the untreated trees. Another article (14) "Uber die innere Therapie der Pflanzen" explains his work in more detail. The two methods of injection used are explained. One of them consists of inserting the dry salt in holes bored in the tree trunk. These holes are then closed with grafting wax. In the other method solutions are injected. The hole made in the trunk for the purpose of injecting is bored with a brace and bit which passes through a metal tube embedded in the tree. A side outlet in this tube is connected by a rubber tube with a jar containing the solution to be injected. As the hole is bored by the brace and bit the solution passes into it, thus shutting out the air from the wound. Diseased trees were injected with copper sulphate, calcium cyanide, and arsenic in 1/100 percent concentration, with inconclusive results. Iron sulphate in 0.05–0.25 percent solutions (amount injected not stated), or the dry salt, 12 grams for trees with 16–25 cm. diameter, cured apple trees of disease and insects. Mokrjetsky stated that he was carrying on more experiments as he believed that the fertilization of plants with such injected salts often cured them at the same time of diseases.

The best reports on tree injection so far printed are the Russian. Most of the experiments were made in the Crimea. Here many of the fruit trees appear to suffer from malnutrition, according to Mokrjetsky (14), and the iron sulphate appeared to act as a most efficient fertilizer. The dry, hot summer climate of this region favored the rapid consumption and transfer of the injected solutions, to which the trees reacted in a striking manner. No reports have been found as to the length of time the injected iron sulphate acts as a fertilizer, except a statement by Mokrjetsky that in the spring following the injection the buds on the fruit trees were numerous and large. The Russian experimenters appear to have stopped, unfortunately, before they had concluded their work. In 1912 the writer received a letter from Shevyrev saying that he was unable to continue the injections and hoped that the work would be carried on in this country.

A series of short papers by German, French, and American workers followed Shevyrev's publications.

Roth (15) in 1896 described a method and apparatus for injecting trees.

Mangin (16) in 1898 unfavorably criticized plant injection, especially the idea that grape vines could be protected from fungi by the injection of salts. He regarded plant injection impracticable in agriculture.

Goff (17) found the injection of water into the roots of newly transplanted trees to be beneficial. He described his apparatus and method of injection. His experiments showed that this treatment hastened the initial growth of the trees.

Bolley (18) in three reports described experiments in stimulating tree growth by injecting liquid solutions into the trunk. He successfully treated diseased apple and plum trees with a formaldehyde solution of 1/2 to 2 parts per 1000 of water. He reported that the effect of injected solutions on parasitic diseases was inconclusive.

Simon (19) reported that he successfully injected apple and peach trees, grape vines, and potatoes. Water solutions of purin and potassium nitrate and nutrient solutions were used. Copper sulphate injected into grape vines was at first injurious, but later the vines produced new leaves free from fungi.

Fron (20), using Simon's method of injection, treated pear trees with solutions of iron sulphate and calcium nitrate. The vigor of the trees appeared to be increased, but the improvement was confined to portions of the trees only. He believed this method of little practical value in fruit culture.

Coffigniez (21) experimented about the same time with iron sulphate and fruit trees in the control of fungus diseases.

Sanford (22) published a note on the effect of potassic cyanide on the scale. He considered the insertion of the salt beneficial to the tree. This result was disputed by Surface (23), Shattuck (24), Moore and Ruggles (25), and Flint (26). The experiments of the latter-named workers showed the injurious effects of a concentrated solution of cyanide of potassium on plant tissues. No attempt was made to try the effect of a gradual impregnation with dilute solutions of the salt. These articles are reviewed by Elliott (27) in a publication which describes the effect of cyanide of potassium on woody and herbaceous plants. Elliott worked with a killing solution, as he inserted the crystals under the bark and epidermis of the plants and depended on the sap to dissolve the crystals. The reactions of the plants were extreme, the tissues in the path of the solution being killed when the solution was concentrated. He found that the weather had a decided effect on the kind of reaction and the time of response of the tree. Trees treated on cool, damp days responded more slowly and showed less extensive injury than those treated on hot, dry days. He found also that the rate of transpiration affected to some extent the path of the solution. When transpiration was slow the solution passed into the cells surrounding the vessels; when it was rapid the solution appeared to pass through the vessels without going into the surrounding cells.

Rankin (28) injected ten chestnut trees with lithium nitrate solutions varying from 0.1 to 0.002 percent. His analysis of the trees showed that the salt had penetrated the bark and sapwood above and below the place of injection. When trees were less than three inches in diameter there was complete penetration of the heartwood, but in trees of greater diameter the penetration did not seem to follow a definite rule, the heartwood sometimes being impregnated, sometimes not. The tip of the trees was found impregnated. Aside from blotching of leaves the trees were not injured.

The Russian and American papers give the most definite reports, both as to practical methods of injecting and as to the results of the injection.

The Problem

In studying such a problem as the injection of a tree, a number of fundamental considerations present themselves:

A substance in solution injected into a tree generally passes through those vessels in the neighborhood of the place of injection through which the crude sap ascends from the roots to the leaves. It can also descend through those vessels, but in all of this there is lacking that persistent passing and returning of a stream such as constantly bathes the cells of the animal body.

The streams passing through this region, besides varying constantly in rate of flow, content, concentration, and acidity, are also under different atmospheric pressures.

The physical attributes of the cells must be considered. The surface of the cell walls, aside from the semipermeable membranes of the living cells in the region of the vessels, offer surface films which are constantly within the field of absorptive and adsorptive forces.

The chemical content of the sap may be changed by the injections, insoluble mineral compounds may be formed and toxins made harmless thereby.

These conditions at this stage of experimentation called for a great deal of empirical experimental work with chestnut trees.

In order to study this subject fundamentally, an attempt was made to answer by means of experimentation the following questions: (1) What substances can be injected into living chestnut trees? (2) When can they be injected? (3) Where does the injected material go? (4) What is the effect on the chestnut tree? (5) What is the effect on the fungus growing parasitically on the trees?

The present record gives the results of five years' experimental work. The work here reported is not complete. The propositions offered for solution have, however, been so varied in character that it seemed proper to bring together in this and a succeeding paper the different results so far secured, since this work must for the present be laid aside.

EXPERIMENTAL PROCEDURE

Experimental Plots

The principal experimental plots of trees were in the center of a blight-infected chestnut orchard of some three hundred-odd acres' extent, located in southeastern Pennsylvania. They were on top of a hill about 500 feet above sea level. This region is hilly and originally was covered by a mixed forest of conifers and deciduous trees, a large proportion of the deciduous trees being chestnuts. The fact that this is the fourth generation of chestnut trees growing here since the Revolutionary War shows how favorable is this region to the growth of chestnut.[2]

Trees

The trees used in the experiments were orchard trees, for the most part Paragon scions grafted on native chestnut stock, *Castanea dentata*. The trees in the plots varied in age according to the year of grafting. One set was about ten, the other fourteen years old. They were short, stocky trees

---

[2] An analysis for alkali content was made of the soil by the Bureau of Soils, Department of Agriculture.

K_2O	trace	
CaO	0.27%	No CO_2 from carbonates.
MgO	0.68%	
P_2O_5	trace	
N	0.08%	
Li	none	

in form, the greatest height being about five meters, the mean height four meters. The orchard had never been pruned or cared for other than by cutting out the underbrush just before the chestnut harvest each fall.

In 1912, when the plots were chosen, they were cleared of underbrush and dead infected trees and were kept clear. Such cankers as threatened soon to girdle the trees were cut out under sanitary conditions. The remaining cankers on the trees were outlined with paint in order to note their rate of growth. The apparatus used in making the injections has been described elsewhere (29)[3].

Injections

Generally two injections were first made in a tree, on opposite sides of the trunk. The next two injections were at right angles to the first two, a little higher up the tree. If more injections followed they were made still higher up in the spaces between the first injections, or on the branches. Observations on the trees injected with substances which blotched the leaves showed that in this way all the branches on the tree could be reached. The hole cut for injection was one centimeter in diameter, and the width of two annual rings of wood into the tree's interior. All the records are based on the intake through holes of this size.

All the substances injected were dissolved in water. This water came from a spring in the orchard and was very lightly mineralized.[4]

---

[3] In 1915 a different method of injecting trees was tried. In place of the clamps used in the old method, link chains tightened by turnbuckles hold the perforated rubber corks against the tree trunk. The corks are protected from the metal chain by iron washers. Glass T-tubes thrust through the corks introduce the salt solution into the injection holes. The tubes leading from the reservoirs are attached to the vertical ends of the T-tubes. The free ends of the horizontal arms of the tubes are tipped by pieces of rubber tubing. A tempered steel tube shaped like a laboratory cork borer makes the holes in the trunk. It can be driven into the tree through the horizontal arm of the T-tube after the apparatus is in place and the solution fills the T-tube. The solution is cut off by a pinch cock placed over the end of the rubber tip after the drill has been removed. Glass T-tubes were found to be safest for this work because the presence of air bubbles, or leakage in the connections, could be detected easily. It is necessary, for a good injection by this method, that no air enter the injection hole. Seven injections at a time have been made by this method.

[4] Analysis of water by Bureau of Chemistry, Department of Agriculture:

	Mg. per liter
Silica (SiO_2)	5.8
Sulphuric acid (SO_4)	0.8
Bicarbonic acid (H_2CO_3)	10.4
Nitric acid (NO_3)	0.5
Chlorine (Cl)	1.5
Iron (Fe)	0.2
Aluminum (Al)	0.0
Calcium (Ca)	1.2
Magnesium (Mg)	0.9
Potassium (K)	0.7
Sodium (Na)	2.0

The water was tested for heavy metals, lead, copper, etc., none being found.

Measurement of Intake

The intake of an injected tree was measured by weighing the jars containing the solutions. This was done with a small brass beam-balance which recorded the weight in grams. It was assumed that a cubic centimeter of the solution weighed a gram. It was thought that the amount of error caused by this assumption was so small as not to need to be calculated when estimating the amount of substance injected into a tree. Experiment showed that the amount of evaporation from the jars through the parchment covering was so small that it could be ignored. This amount was found to average 40 cc. per month. If the paper cap was torn the average evaporation was 70 cc. per month.

There was also evaporation of the more volatile substances in dilute solution. This could be noticed in the case of the cresols and phenols and of some of the ammonium solutions. The amount of this loss was not tested. The jars containing such solutions had their contents renewed frequently, and an attempt was made by reinjecting to keep the solutions going into the trees rapidly. These precautions were thought to be sufficient to make it unnecessary to calculate either the loss or the concentration of substance due to such evaporation in the experimental work so far attempted.

Substances Injected

The following substances were injected into the trees:

Inorganic Substances

Copper sulphate
Copper chloride
Zinc carbonate
Mercuric chloride
Potassium chromate
Potassium bichromate
Barium chloride
Colloidal cuprous hydroxide[5]
Colloidal metallic silver
Colloidal metallic mercury
Potassium carbonate
Potassium hydroxide
Potassium sulphate
Ammonium carbonate
Ammonium chloride
Ammonium hydroxide
Ammonium sulphate
Sodium carbonate

Organic Substances

Methyl alcohol
Formalin
Acetic acid
Formic acid
Lactic acid
Citric acid
Aniline sulphate
Phenol
Sodium carbolate
Phenol Sodique[6]
Para nitro phenol
Ortho nitro phenol
Picric acid
Meta cresol
Para cresol
Thymol
Pyrocatechin
Pyrogallic acid

---

[5] I am indebted to H. K. Mulford Company of Philadelphia for these colloidal preparations. The metals were protected in each case by a second colloid.

[6] A patent medicine made of carbolic acid and caustic soda.

Sodium chloride
Sodium hydroxide
Lithium carbonate
Lithium chloride
Lithium sulphate
Lithium hydroxide
Lithium nitrate

Water

Phloroglucin
Oil of bitter almonds
Benzoic acid
Salicylic acid
Bark extracts
Water extract of chestnut tree bark
Water extract of chestnut blight canker

Stains:

Methyl green
Methylene blue
Eosin
Congo red
Trypan blue

All these substances went into the trees in measurable quantities.

Solutions

The solutions were made *gram molecular* except in the case of stains, the bark extracts, formalin, Phenol Sodique, and ammonium hydroxide.

For instance, if a solution of anhydrous sodium carbonate 1/200 G. M. is used, the molecular weight of sodium carbonate is found, which is 106.10. 106.10 grams of salt added to a liter of water makes a gram molecular solution, and a solution 1/200 G. M. means that 1 cc. of the G. M. solution is added to 199 cc. of water.

The chemicals used were bought as chemically pure.

But one substance was injected into a single tree. In a few cases, all of which are indicated in a following list, stronger solutions were used in the later than in the earlier injections in a tree.

Number of Trees Injected

Usually three or more trees were injected with the same substance. The exceptional cases in which fewer than three trees were injected are as follows: But one tree injected: methyl alcohol, Phenol Sodique, oil of bitter almonds, and para cresol. But two trees injected: zinc chloride, barium chloride, colloidal metallic silver, and colloidal metallic mercury. The largest number of trees injected with one salt was thirteen, injected with lithium carbonate solutions of different dilutions. Nineteen check trees were injected with water.

Some of these trees were injected two years in succession, some three years, the greatest number but one year.

The injections were made in 1912, 1913, and 1914. In 1913 a record of the weather was kept together with a record of the daily intake of the trees, so that all remarks on the rate of intake of the trees will be confined to the records of this year. The records of the previous and succeeding years confirm the 1913 figures.

RATE OF ABSORPTION OF INJECTED SUBSTANCES

This compilation was made from the records of the injections made in 156 Paragon chestnut trees during the growing season of 1913 and of the weather during that period.

The therapeutic bias of the work decidedly limited the scope of the experimental injections, and in consequence data are wanting for a complete record of the rates of intake. The effort was to find the dilution at which a substance entered a tree readily without killing it. When a tree showed injurious effects of an injection, the injection stopped whether it had been going for two days or for a week.

It was the policy in 1913 to inject large quantities of dilute solutions, on the supposition that the dilution decreased the toxicity of the substance near the point of injection. At the same time the tendency of the lignified cell walls to retain the substance was relied on to dilute the solution still further in its passage toward the leaves, so that the latter would not accumulate so much before autumn as to cause them to die. In consequence of this effort the data on the rate of absorption are very incomplete.

TABLE 1. *Substances, with Their Dilution, Injected Into Trees*

No. of trees	Substance	No. of trees	Substance
	Ammonium:		
4	$(NH)_2CO_3$ 1/100 G.M.	3	Acetic acid
4	$(NH_4)OH$ 1/100 approximately		(2) 1/1000 G.M.
6	$(NH^4)_2SO_4$		(1) 1/3000
	(1) 1/100, changed later to 1/50	3	Benzoic acid
	(2) 1/100		(1) 1/1000
	(2) 1/200		(2) 1/5000
	(1) 1/500	3	Citric acid
2	NH_4Cl 1/200		(1) 1/500
	Sodium:		(1) 1/3000
7	Na_2CO_3		(1) 1/5000
	(4) 1/100	3	Formic acid
	(2) 1/200		(1) 1/1000
	(1) 1/500		(2) 1/6000
4	$NaCl$	3	Lactic acid
	(3) 1/100, changed to 1/50		(2) 1/1000
	(1) 1/200, changed to 1/50		(1) 1/2000
7	$NaOH$ 1/100 G.M.	4	Picric acid
	Lithium:		(1) 1/500 G.M.
5	$LiOH$		(1) 1/1000
	(1) 1/200		(2) 1/10000
	(3) 1/500	2	Pyrogallic acid
	(1) 1/1000		(1) 1/100
5	Li_2C		(1) 1/1000
	(1) 1/200	3	Salicylic acid

(1) 1/500, changed to 1/100
(1) 1/5000, changed to 1/1000
(1) 1/1000, changed to 1/500
(1) 1/100
4........LiCl
(2) 1/100
(2) 1/200
Potassium:
4........KOH
(2) 1/100
(2) 1/200
4........K_2CO_3 1/100
4........K_2SO_4 1/100
5........Colloidal copper
(5) 1/3300
2........Colloidal metallic silver
(2) 1/6400
2........Colloidal metallic mercury
(2) 1/6400
3........Potassium bichromate
(1) 1/1000
(2) 1/5000
5........Potassium chromate
(1) 1/5000
(1) 1/1000
(3) 1/1000
1........Copper sulphate
(1) 1/100
(2) 1/5000
(1) 1/10000
3........Aniline sulphate
(3) 1/1000
3........Meta cresol
(3) 1/1000
1........Para cresol
(1) 1/1000
4........Ortho nitro phenol
(2) 1/1000
(2) 1/100000
3........Para nitro phenol
(2) 1/500
(1) 1/1000
1........Oil of bitter almonds
(1) 1/1000
1........Phenol Sodique
(1) 1 cc. to 1,000 cc. H_2O
3........Phloroglucin
(3) 1/1000
3........Pyrocatechin
(1) 1/500
(2) 1/1000
3........Sodium carbolate
(3) 1/1000
3........Thymol
(1) 1/1000
(2) 1/3000
1........Methyl alcohol
(1) 1/100
2........Methylene blue
(2) 1 gm. to 4,000 cc. H_2O
2........Trypan blue
1 gm. to 4,000 cc.
Bark extracts:[7]
3........Healthy bark
(3) 1 cc. to 99 cc. H_2O
2........Canker extract
(2) 10 cc. to 990 H_2O
3........Canker extract-citric acid
(3) Canker ext. 1 cc. to 100 cc. H_2O, with citric acid 1/500 G.M.
13........Water checks

The records of absorption were divided into months for convenience in tabulating. It was found that injections could be made in February and March, when the rate of intake was very slow. The regular injections began in April, but the records for this month were not typical because it was not until the latter part of the month that injection and weather-recording apparatus were in running order.

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[7] The bark extracts were made by soaking for 24 hours the shredded bark and young wood in spring water. The proportions were 10 cc. of water to 1 g. of healthy bark or of canker tissue. The extracts were filtered before using.

The daily intake of the trees was measured each morning, and usually the injections were made in the morning. The hourly intake was not measured, but experience confirmed Shevyrev's observations that the intake by day was greater than by night.

The records of series of injections in individual trees showed that the number of the injection did not influence the amount of solution which went into the tree, *i.e.*, at the sixth injection more cubic centimeters might go into the tree than at the first, or the third injection in the month might be more successful than the first or second. As has been explained, (page 7), care was taken that the new injection was not made directly above or below the old injection hole.

The intake of the trees in the different months was computed and plotted on ruled paper in order that estimates of the rates could be made.

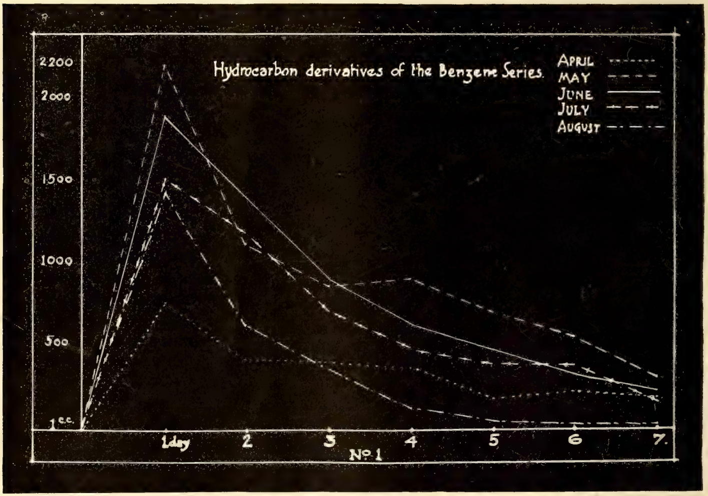

FIG. 1. Graph showing the rate of intake of trees injected with the hydrocarbon derivatives of the benzene series during the spring and summer months.

In computing, the *mean* of the intake of all the injections of a tree during the month represented the monthly intake of that tree per injection.

Plotted curves showing the rate of intake are more varied for April, May, and June than were those for the summer and autumn months. Figures 1, 2, and 3 show the *mean* intake a day per tree reckoned from the day of injection for seven days, of all the trees injected with alkali metals, organic compounds, and water. As the number of trees being injected varied from month to month, these curves simply approximate the rate of intake.

93 trees are represented in the curves of the hydrocarbon derivatives of

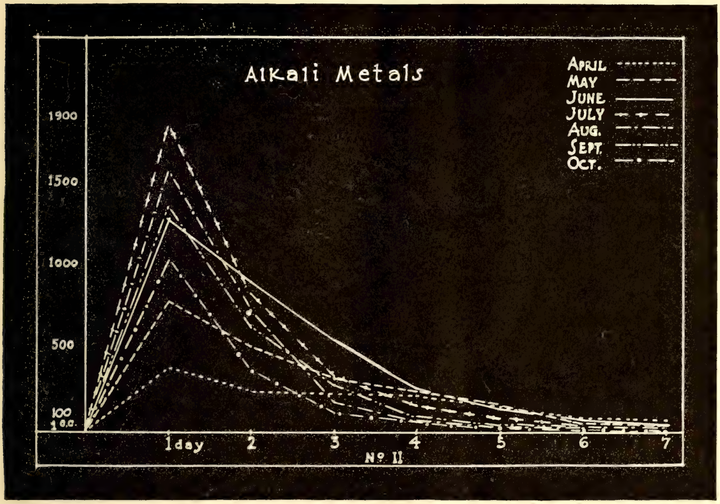

FIG. 2. Graph showing monthly rate of intake of trees injected with the alkali metals.

the benzene series: 7 trees in April, 17 trees in May, 26 trees in June, 33 trees in July, and 10 trees in August. The curve for May, for instance, represents more than 17 injections, for, as has been explained (p. 7), two injections were made in a tree on opposite sides of the trunk, and the daily amount of intake of an injected tree was the mean intake through two injection holes. In May it happened that many of the injections continued for two and three weeks. (The more readily the solution flowed into the tree the fewer were the reinjections.) A number of the trees were injected during one month only, very few for three months, so that no comparison between the intake of a solution by a single tree in the different months could be made. For these reasons the curves approximate the rate of intake, as has been previously stated.

The curves representing the alkali metals are better representations because more trees (121) are represented: 9 in April, 8 in May, 9 in June, 26 in July, 33 in August, 30 in September, and 17 in October. Not only are more trees represented, but more injections to a tree. In spite of the large number of trees, the curves for April, May and June are not typical, being depressed by the ammonium solutions (counted with the alkali metals), which were injected at this time when comparatively few trees were being treated.

With hardly an exception the rate of intake for the solutions, irrespective of whether they were acid, neutral, or alkaline in reaction, was greater than for water. The exceptions were weak solutions of the ammonium compounds, formic acid 1/6000 G. M., chestnut bark extract, canker extract, and possibly the colloidal solutions of metals.

The typical curve of intake reached its highest point the first 24 hours after injection, then decreased steadily.

Figure 4 shows the rate of intake of an equal number of trees injected July with acids, alkalies, and water. The alkalies surpassed the acids in the first 24 hours, but in the second they dropped one half in quantity, and continued to decrease more rapidly than the acids. Because of this rapid decrease in the daily intake of the alkali metals, the trees treated with these compounds usually were injected once a week.

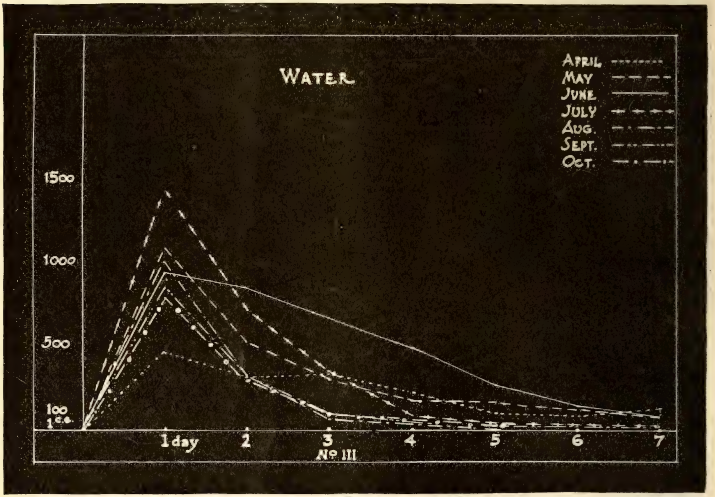

FIG. 3. Graph showing monthly rate of intake of trees injected with water.

Rankin (28) obtained somewhat similar results when injecting chestnut trees with solutions of lithium nitrate, *i.e.*, the greatest intake was during the first two days and had practically ceased after the fifth and sixth days.

The injections of carbon compounds often ran for three weeks without a reinjection, sometimes longer. The most marked example of this readiness of intake was a tree injected with para nitro phenol (1/1000 G. M.). This solution flowed into the tree steadily for 41 days without a reinjection. In this time 32½ liters went into the tree through two holes each one centimeter in diameter.

The rate of absorption of the solutions of organic compounds was much greater than the rates of absorption of the solutions of the alkali metals, the heavy metals, and water.

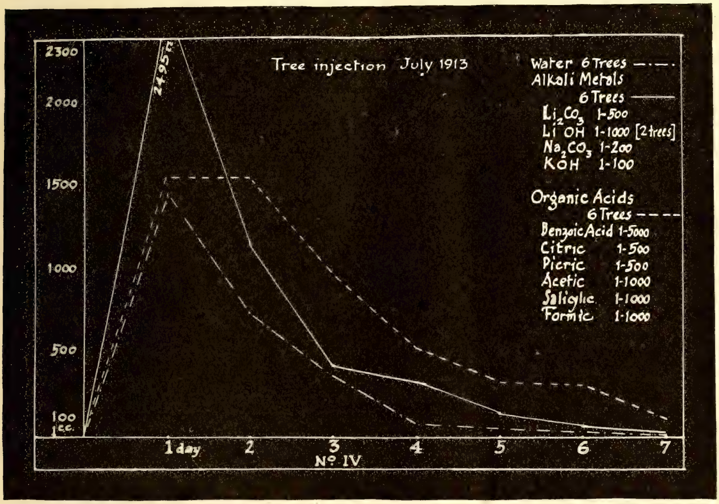

FIG. 4. Graph comparing rate of intake of trees injected during July with water, alkali metals, and organic acids.

The daily intake of the carbon compounds was extremely irregular. Sometimes the curves seem to indicate that for a short period the intake measured variation in the transpiration of the trees.

The curves of intake of a single tree injected with LiOH 1/200, and those of 3 trees with LiOH 1/500, represented in figures 5 and 6, show how regular was the daily intake of the trees injected with alkali metals. These diagrams also illustrate the fact, common to all the chemicals injected in these experiments, that the greater the concentration of the solutions the greater the intake.

The colloidal solutions of metals were injected into small trees in April before the leaves appeared. All the solutions went in slowly but steadily.

The healthy bark extract went into the trees more readily than the canker extract. An addition of citric acid to the canker extract increased the intake. These extracts were injected in April and May.

The rate of absorption of solutions of the heavy metals approximated that of solutions of the alkali metals. A solution so concentrated as to be deadly entered the trees more readily than did the more dilute solutions.

During the treatment of the trees a daily record of the weather was kept by means of standard instruments. Some of the weather recording apparatus was not set up until the latter part of April. But after April the records were kept until work stopped the last of October. A detailed account of the evaporation and rainfall for this season is given elsewhere (30).

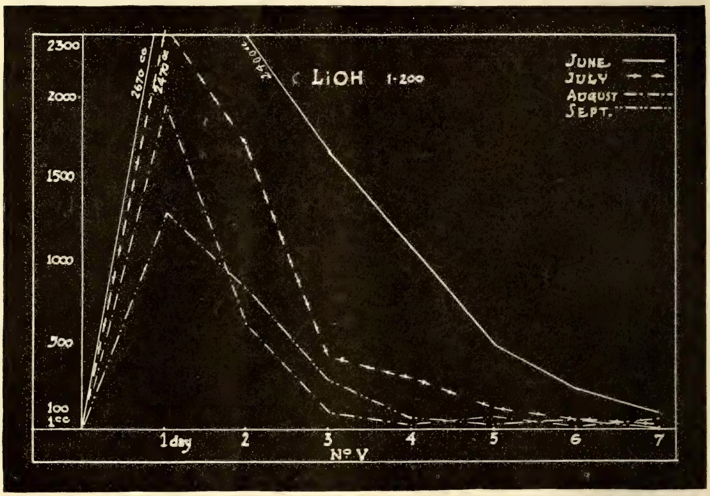

FIG. 5. Graph showing rate of intake of trees injected during the summer months with lithium hydroxide 1/200 G.M.

In 1913 the growing season of the chestnut began on April 28, when the leaf buds opened. In May the leaves were nearly mature in size, and flower tassels appeared. By June the leaves were full grown, the flowers had blossomed, and the fruit had set. In July the burs on the trees were half-grown, in August full-grown. In September the nuts began dropping. In October nuts, burs, and leaves dropped from the trees.

Figure 7 shows a monthly compilation of the weather records and of the amount of solution absorbed by a tree per day during each month, every tree injected during the season being used in the computation. The figures in the monthly weather records represented the *mean* of the daily

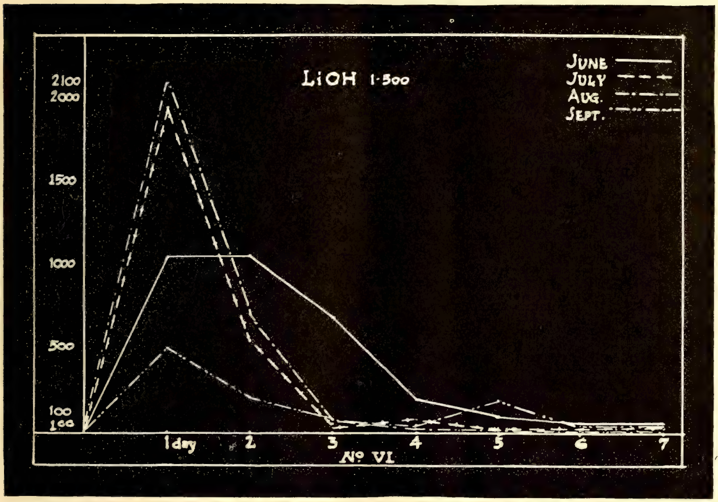

FIG. 6. Graph showing rate of intake of trees injected during the summer months with lithium hydroxide 1/500 G.M.

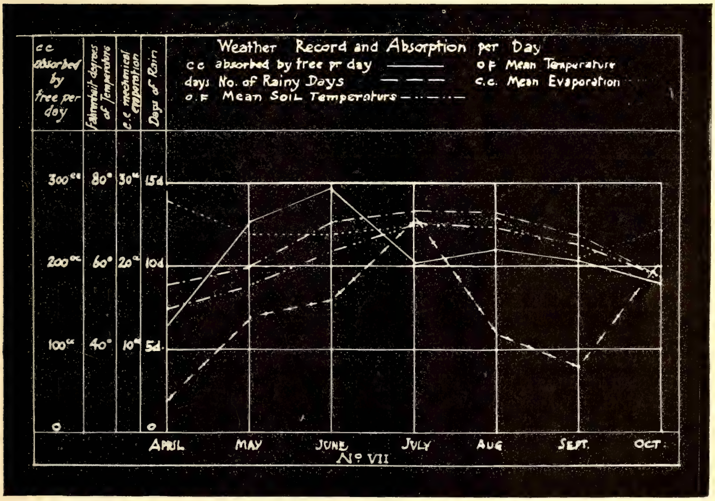

FIG. 7. Graph showing the monthly compilation of the weather records and the amount of solutions absorbed by the trees injected during the year 1913.

records for that month. The amount of rainfall[8] is not recorded in the diagram, because it was noticed that the number of inches of rain which fell on the hill was not so influential in so far as the injections were concerned as was the number of rainy days.[9]

The diagram shows the considerable capacity of the chestnut tree for absorbing chemical solutions.

The *mean* amount absorbed by a tree per day in a 7-day-long injection, was in April 103 cc.; in May 255 cc.; June 299 cc.; July 201 cc.; August 229 cc.; September 224 cc.; and in October 178 cc.

Comparing the records of the intake of the trees with the weather records, it can be seen that the amount of intake is dependent on the stage of development of the trees, which in turn is dependent on the periodic change of weather during the season. The greater the capacity for transpiration, the larger the initial amount of intake. The irregularities of the curves are due to transient changes of weather modified in turn by the changing capacity for transpiration.

From these records of 1913, it appears that the most favorable month for injection of chestnut trees, so far as rate of intake is concerned, was June; after this month came, in rank, July, May, August, September, October, and April.

SUMMARY

A compilation of the records of injections made in 156 Paragon chestnut trees during the growing season of 1913 shows that the trees possessed a considerable capacity for absorbing solutions of substances.

June was the best month for injection in so far as rate of intake was concerned, then came July, May, August, September, October, and April. The rate of intake varied more in April, May, and June than in the summer and autumn months.

Solutions of organic compounds went into the trees more readily than solutions of inorganic compounds, the "true solutions" more readily than the colloidal.

Injected solutions, with a very few exceptions, were absorbed more readily than injected water.

The more concentrated the solutions of chemicals were, the more readily they were absorbed by the trees.

The effects of the injections here described upon the trees and upon *Endothia parasitica* will be discussed in a later paper.

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[8] The rainfall during the period of injection work was 24.7 inches.

[9] For example, in July only 3.1 inches of rain fell, but there were 13 rainy days, the amount of solution absorbed per tree per day dropped during July to 201 cc. In May 4.5 inches of rain fell, with 7 rainy days; the absorption per tree per day was 255 cc. In August 5.8 inches of rain fell with 6 rainy days, and the absorption per tree was 229 cc. per day.

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THE OCCURRENCE AND INHERITANCE OF SEX INTERGRADATION IN PLANTS

CECIL YAMPOLSKY

In a previous paper (1919) I have called attention to sex intergradation in *Mercurialis annua* in both male and female cultures. It is my purpose here to discuss the general question of sex intergrades as they occur in the flowering plants. This discussion is based in a large measure upon the results reported in the paper mentioned above.

There can be no question from the data at hand that sex in Mercurialis is a fluctuating rather than a fixed character expressing itself in a wide range of sex intergrades, including as the extremes some pure male and some pure female plants and midway between the extremes highly fertile monoecious forms. The sex intergrades here are all highly and equally fertile, and no suspicion of abnormality or of pathological conditions can attach to them. That there is a tendency to pure dioecism seems highly probable, but the transition from hermaphroditism is still represented by all possible gradations, showing most convincingly that theories of sex determination based on the segregation of fixed unit factors can have no significance for such types.

SEX INTERGRADES

Goldschmidt (1916*a*) reports in a preliminary paper upon the sex ratios in crosses between the European and Japanese races of the gypsy moth, *Lymantria dispar*. He obtains various gradations in the sexual condition unlike the well known gynandromorphs. His individuals do not, as in the case of the gynandromorphs, show a sectorial arrangement of the characters of the two sexes, but they do show different gradations between the extremes of femaleness and maleness. His females show all the transition stages, such as feathered antennae, male wing pigmentation, the transition of ovaries into testes, and the loss of the power to lay eggs. His males show tendencies towards femaleness in a similar manner. For these individuals he proposes the term *intersexes*. He finds that as his sex intergrades approach the middle line between maleness and femaleness, they become more and more sterile, that is he obtains no fertile hermaphrodites such as occur for example in *Mercurialis annua*. In fact, although his forms show morphological intersexualism, they are functionally sexless in many instances. To be sure, he secures his intergrades by using as parents forms which in themselves possess functional sexual intergradation. As noted in my previous paper, there is a tendency in plant forms that exhibit gradations in sex (judged by the proportion of male, female, and hermaphroditic flowers) to show a definite influence of that condition upon the sex ratios in the offspring.

Banta (1916) reports the appearance of intersexes in a phyllopod, *Limocephalus vetulus.* The females reproduce parthenogenetically. "In one of the strains there appeared a large percentage of males together with normal females and a large number of sex intergrades—males with one or more female secondary sex characters, females with one to several male characters, and some hermaphrodites with various combinations of male and female secondary sex characters." Eight secondary sex characters distinguish the male from the female. The highly male-like female intergrades are relatively infertile. The more the female takes on the male characters the less likely she is to be fertile. Some individuals with several secondary male characters prove to be very fertile. "In general in addition to being more prolific one may say that female intergrades with few or less distinctly male characters produce a smaller percentage of males and sex intergrades than those having a larger number of more definitely male characters." Males that show one or more female secondary sex characters nearly always have an incompletely developed reproductive system. By propagating from female intergrades, Banta was able to secure the production of mixed broods, males, females, and sex intergrades. The stock derived from these females consists of 40 percent normal males, 8 percent normal females, and the rest intergrades with almost any combination of male and female secondary and primary sex characters. Some of his sex intergrades (female) may parthenogenetically produce normal females and occasionally normal males.

In a later paper, Banta (1918) reports on sex intergrades in *Daphnia longispina.* In this form the male differs from the female in eight secondary sex characters. In Daphnia there are fewer male than female intergrades. The offspring of the more highly male female intergrades tend to be like the mother. A female from a sex intergrade will produce offspring very much like herself with few male secondary sex characters. The more male the female intergrade, the more sterile she is likely to be.

Banta makes the following suggestive remark: "From such clear cases of sex intermediates one wonders if maleness and femaleness are really mutually exclusive in those Cladocera individuals which morphologically show no unlike sex characters. Even in 'normal' strains one is certainly justified in thinking that maleness and femaleness are not complete and mutually exclusive states but that in these apparently normal sex forms, too, sex is also relative—differing from so-called sex intergrades not in kind but merely in degree, not qualitatively but quantitatively."

Plants show most clearly that maleness and femaleness in the same individual do not tend to neutralize each other and to produce sterility. The appearance of intersexes or sex intergrades in the plant kingdom, while not designated by these terms, has been described for very many forms in the phanerogamic floras. A definite terminology is used in botanical literature to cover this phenomenon. The terms *hermaphroditic*, *dioecious*, *monoecious*, *andromonoecious*, *gynomonoecious*, *trimonoecious*, *gynodioecious*, *trioecious*, *androdioecious*, etc., are used.

A hermaphroditic form is one in which both pistils and stamens are borne in the same flower (perfect flower), and in which all the flowers on a plant show the same arrangement of parts. Example: Lilium.

A dioecious form is one in which the sexes are completely separated so that one plant bears male flowers only and the other plant female flowers only. Example: *Elodea canadensis.*

A monoecious form is one in which the pistillate and staminate inflorescences are borne separately on the same plant. Example: Begonia.

An andromonoecious form is one that bears perfect flowers and male flowers on the same plant. Example: many Umbelliferae (Lotsy, 1911).

A gynomonoecious form is one that bears perfect flowers and female flowers on the same plant. Example: Atriplex and many Compositae (Lotsy, 1911).

A trimonoecious form is one that has three distinct types of flowers, male, female, and hermaphroditic. Example: *Acer campestre* (Lotsy, 1911).

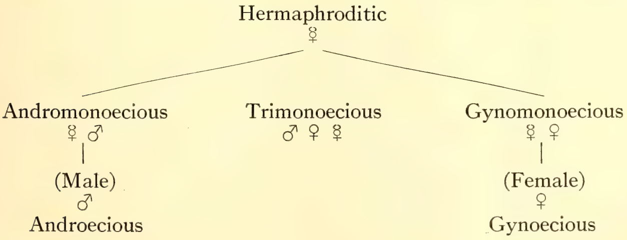
A gynodioecious form is one that has both female and hermaphroditic individuals. Example: *Plantago lanceolata.*

An androdioecious form is one that has both male and hermaphroditic individuals. Example: *Caltha palustris* (Lotsy, 1911).

Other combinations may occur. Staminate and pistillate inflorescences may appear on one individual and hermaphroditic on another. Example: Callitriche (Lotsy, 1911). Male and female inflorescences may appear on one individual while another individual may be male. Example: Ceretopus (Lotsy, 1911). Male and female inflorescences may appear on one individual while another may have only female flowers. Example: Morus (Lotsy, 1911). Hermaphroditic and male flowers may appear on one individual while another is female only. Example: Gleditsia (Lotsy, 1911).

A trioecious form is one in which three distinct sex types occur, male, female, and hermaphroditic. Example: hemp.

Correns (1913) offers the following diagram to show these conditions.



He proposes to call these forms *mixed in sex.* The old term is *polygamous.* I have used the term *intersexes* or *sex intergrades.*

These groups indicate in every case the distribution of functional sex elements. We find, however, just as Goldschmidt found in his intersexes, so here, that every possible degree of functional and structural perfection and degeneration exists and has been long known, though botanists have been inclined to take them as matters of course until the development of the recent efforts to make sex determination a matter of absolutely alternative inheritance and to represent sex characters by fixed and invariable factors in the germ plasm which are segregated in the reduction division. We can classify the grades of development of female sex organs as follows. In each case functional stamens are present in the same flower or elsewhere on the same plant.

I. Functional Variation.
  a. Ovaries fertile to pollen from another genus: *Zea Mays* L. × *Euchlaena Mexicana* Schrad.
  b. Ovaries fertile to pollen from one or more different species: *Antirrhinum molle* × *A. majus.*
  c. Ovaries both cross- and self-fertile: *Zea Mays.*
  d. Ovaries cross-fertile and self-fertile: *Pyrus malus.*
  e. Ovaries normal in appearance but both cross- and self-sterile: Eschscholtzia.
II. Structural Variation.
  a. Ovaries normal in size, all ovules functional: *Lilium canadense.*
  b. Ovaries normal in size, ovules more or less aborted: Phaseolus.
  c. Ovaries normal in size, all ovules aborted: *Trifolium pratense,* Syringa hybrids.
  d. Ovaries visibly degenerate: *Fraxinus excelsior.*
  e. Ovaries mere rudiments: *Fraxinus excelsior.*
  f. Ovaries becoming stamen-like: *Salix caprea.*
  g. Ovaries not present, replaced by petals: Matthiola.

In the same way we can classify the grades of development of the male sex organs. In each case functional ovaries are present in the same flower or elsewhere on the same plant.

I. Functional Variation.
  a. Pollen capable of fertilizing ovaries of another genus: *Zea Mays* × *Euchlaena Mexicana* Schrad.
  b. Pollen capable of fertilizing ovaries of another species: *Antirrhinum molle* × *A. majus.*
  c. Pollen both cross- and self-fertile: *Zea Mays.*
  d. Pollen cross-fertile and self-sterile: *Pyrus malus.*
  e. Pollen normal in appearance but both cross- and self-sterile: Eschscholtzia.
II. Structural Variation.
  a. Anthers normal in size, all grains functional: *Lilium canadense.*
  b. Anthers normal in size, not all pollen grains functional: Phaseolus.
  c. Anthers normal in size, some pollen grains aborted: Oenothera hybrids.
  d. Anthers visibly degenerate: *Thymus vulgaris.*
  e. Anthers mere rudiments: *Echium vulgare.*
  f. Anthers becoming pistil-like: *Salix caprea.*
  g. Anthers not present, replaced by petals: Matthiola.

Further, as the classification shows, a great many plants exhibit gradations between maleness and femaleness and hermaphroditism because of the more or less nearly complete degeneration or modification of parts. A female may arise from a hermaphrodite through the more or less complete suppression or degeneration of stamens. Likewise a monoecious individual may become female by the degeneration or the entire suppression of the stamens. A male may arise from either one of such forms by the disappearance or more or less nearly complete degeneration of the carpels. When in a group of hermaphrodites the stamens of some of the plants are suppressed or degenerated we have a condition of gynodioecism, if the carpels are suppressed or degenerate a condition of androdioecism. When only parts of the plants exhibit the phenomena described we find a multiplicity of combinations. A gynomonoecious individual may arise from a hermaphrodite in which female flowers appear through the suppression or degeneration of the stamens. An andromonoecious individual may arise from a hermaphrodite through the suppression or degeneration of some of the carpels. Gynomonoecism and andromonoecism, just as well as gynodioecism and androdioecism, may appear in various gradations; in the former instance by virtue of the suppression or degeneration of larger or smaller numbers of parts of the plant, and in the latter case because of changes that may occur in larger or smaller numbers of individuals in a group of plants.

The appearance of sporadic female or male flowers on a hermaphroditic plant may bring about a condition of gynomonoecism or andromonoecism without necessitating the degeneration of parts. Thus, also, among dioecious forms the appearance of male flowers on the pistillate plants or of female flowers on the staminate plants gives all possible sex combinations found in plants.

There is still another form in which a so-called polygamous condition may exist. In these cases either the female or male elements, although morphologically perfect, are physiologically, either one or both, functionless. We find also gradations in the degree of sterility of stamens or ovaries of parts of the plant, of the whole plant, or of varying numbers of individuals in a group of plants.

Earlier investigators who have observed pistillody of the stamens, staminody of the pistils, the appearance of male flowers on female plants, etc., considered them monstrosities and grouped them as such. Moquin-Tandon (1841) and Masters (1869) include all such phenomena under teratology. The many cases of this sort reported for plants would suggest that this treatment is by no means adequate.

Wehrli (1892), who reports on a case of the complete transformation of a male catkin of *Coryllus avellana* L., has brought together all the available literature from 1741 to 1892. He lists over 80 distinct species, monoecious, dioecious, and hermaphroditic, in which such modifications of floral parts have occurred. The phenomena he observed include: the appearance of male flowers on female plants, of pistils and stamens in the same catkins, appearance of perfect flowers on male plants, abortion of stamens, pistillody of stamens, staminody of pistils, yearly changes in flowers on a tree (nutmeg), sectorial arrangement of male and female inflorescences in *Pinus alba*, and many more. Wehrli's references and *résumé* are so complete that they have not been repeated here. However, a number of typical examples will be listed from the literature since Wehrli's paper.

Haring (1894) gives an elaborate series of drawings showing various gradations in the transition stages of stamens into pistils and of pistils into stamens in *Salix caprea* L. and *S. cinerea* L. He observes that his work shows the tendency in willows to the greatest plasticity in the structure, form, and sex of the floral organs, including the growing together or the separation of parts, the replacement of one sex organ by that of the opposite sex, and the transition of one sex into the other. The author goes on to say that the phenomena that he has described show the morphological equivalence of the organs of both sexes, in the position of the sex organs no matter whether male or female, in the replacement of the organs of one sex by those of the other, and in the transition of one sex into the other.

In the plant kingdom not only is there a transformation of one sex organ into the opposite but the transmuted organs are quite regularly functional, though sterility of the intergrade organs is not uncommon. Intergradation of sex in plants, if measured in percentages, may be from a fraction of one percent to one hundred percent, in the former case by the pistillody of a single stamen or staminody of a single pistil on a whole plant and in the latter case through the complete alteration of a male plant into a female. Sex intergradation as evidenced by the appearance of one or more parts of the opposite sex on a given plant does not seem to affect the fertility of the plant.

We may note in more detail some of the most carefully studied forms which show these sex intergrades. *Satureja hortensis* is described by Correns (1904) as occurring in three forms: (1) plants with female flowers; (2) plants with hermaphroditic flowers, hermaphroditic flowers with shriveled anthers, and female flowers; (3) plants with hermaphroditic flowers and shriveled anthers, and female flowers. These shriveled anthers indicate a tendency to abortion or infertility of the organs of one or the other sex, paralleling the conditions in Goldschmidt's sex intergrades.

Dimorphotheca pluvialis is trimorphic (Correns, 1913). In an earlier paper (1906) he describes the ray flowers as female, the outer disc flowers as hermaphroditic, and the innermost as male.

Correns (1904) finds five forms of *Silene inflata*: males, females, hermaphrodites, gynomonoecious, and gynodioecious individuals.

Wittrock (1886) describes five different kinds of inflorescences in *Acer platanoides*: (1) individuals exclusively female, (2) individuals whose first flowers are female and their later flowers male, (3) individuals whose first flowers are male and their later flowers in part male and in part female, (4) individuals whose first flowers are male and their later flowers female, (5) individuals exclusively male.

Schulz (1892), on the basis of eleven years' observation of the ash (Fraxinus), recognizes ten distinct forms:

(1) Individuals which bear only male flowers.

(2) Individuals which bear only hermaphroditic flowers.

(3) Individuals which bear only female flowers.

(4) Individuals which bear only male flowers one year and the next year show branches of both male and female flowers.

(5) Males which have certain branches either female, hermaphroditic, or with both kinds of inflorescences.

(6) Individuals which one year bear only female flowers, and the next year have branches with more or less hermaphroditic and female flowers.

(7) Individuals bearing equal numbers of female and hermaphroditic flowers on the same or different branches.

(8) Individuals which bear one year only hermaphroditic flowers and almost always associated with them female flowers, later producing male flowers.

(9) Female or hermaphroditic individuals with male branches.

(10) Individuals with about equal numbers of male, hermaphroditic, and female flowers.

Correns (1908) says that there are at least thirty intergrading categories recognizable in *Plantago lanceolata*. In his classification of forms for experimental purposes he recognizes the following classes: (1) hermaphrodites, (2) predominantly hermaphrodites, (3) hermaphrodites and females, (4) predominantly females, and (5) females.

I have given only a few examples of the very many that are listed in the plant kingdom, but the forms cited are sufficient to show the wide range of intersexuality that exists among plants. These cases of intergrades in functional and structural development of the sex organs, taken in connection with the classes based on the distribution of the sex organs by plants as individuals as tabulated above, present an almost bewildering completeness as a picture of the theoretically possible gradations in sex characters both of the gametes and of the organisms which produce them. And it is to be remembered that for the most part these are not exceptional or chance cases. They represent the common and obvious facts as to sex in the flowering plants. No theory of sex based on the assumption of the alternative inheritance of fixed sex factors which are segregated at the time of the reduction divisions can do justice to the conditions presented in the higher plants.

I have brought together data as to the distribution of sex forms in the various orders of seed plants. For this purpose I have followed Engler and Gilg's "Syllabus der Pflanzenfamilien." Practically every order has families which contain forms that show more than one kind of distribution of the sex elements. Thus in the monocotyledons ten of the eleven orders have hermaphroditic, monoecious, dioecious, and polygamous individuals. There are twenty-two families represented in the ten orders. In the dicotyledons thirty-one of the forty orders have representatives of two or more of the various distributions of the sex elements. There are ninety families that exhibit this tendency. At the end of the paper are listed the families and the sex forms found in each. Their distribution is further shown by means of a table.

Change of Sex Apparently as a Result of Environmental Influences

Changes of sex from year to year and apparently as a result of environmental influences are inextricably interrelated with the fluctuations of maleness and femaleness in sex intergrades and must hence be briefly considered here.

Gallardo (1901) reports on the work of Spegazzini, who by transplanting wild female plants of Dioscorea, Clematis, and Trianosperma found that the following year fruit was set. Examination showed that these plants bore either male or hermaphroditic flowers besides the female flowers. The following year, however, they became female again. Male plants, transplanted, showed no change of sex.

De Vries (1903) figures the appearance of seeds on a male branch of *Mercurialis annua*. Strasburger (1910) cut back 200 male plants to ascertain whether severe pruning would have any effect upon them. Only one male plant that had been cut back produced a single female flower. One of his plants, no. 16, started as a pure female. It began, however, gradually to develop male flowers with functional pollen. It became more and more male, producing the characteristic odor of the male plants. He collected 55 seeds from this plant but only 5 germinated, 2 males and 3 females being produced. This behavior of Strasburger's plant, with reference to the production of a mixed progeny, might perhaps be explained on the basis that the seeds set when the plant was predominantly female produced female offspring, while the seed produced when the plant was predominantly male produced males. The 55 seeds may even have represented the three conditions, male, female, and hermaphroditic.

Higgins (1916) reports a case in which a male plant of *Carica papaya* was cut down, leaving only a stump. This stump sent out branches which bore abundant fruit.

Pritchard (1916) found that by mutilating male and female plants of hemp the appearance of organs of the opposite sex could be induced. The author calls attention to the presence of monoecious individuals as a normal occurrence, often constituting as high as eight percent of the dioecious cultures.

Davey and Gibson (1917) have found in Myrica, which is described as dioecious, gradations in sex like those described for other forms. They find a small proportion of monoecious plants which represent all gradations between the normal pistillate and staminate types. They also describe bushes and shoots whose sex may vary from year to year. Fourteen cases found to be entirely pistillate in 1913 and 1914 produced staminate catkins in 1915. One plant produced almost entirely staminate catkins. Certain trees and branches which produced abundant fruit in 1913 developed mixed shoots in 1914 and in 1915 became almost staminate.

The classic case of alteration of sex in plants is that of *Lychnis dioica* when attacked by the anther smut fungus, *Ustilago violacea*. Strasburger (1900) points out that both male and female plants are attacked by the smut. In the anthers of the male the parasite causes a characteristic purplish color, the interior of the anther being filled with smut spores. In the female the fungus causes a more profound change. The plant is stimulated to produce anthers with the characteristic sporogenous tissue which tissue is later destroyed by the fungus so that the anther is ultimately filled with fungus spores.

Although the list which I have brought together is by no means complete, it is, however, sufficiently representative of the changes in sex that have been reported in the literature. Sex intergrades, it will be noted, may occur in various degrees, from the transition of one sex organ into that of the opposite sex to a complete change of sex of the entire plant.

SECONDARY SEX CHARACTERS

It is to be noted that intersexualism in animals is measured by the degree of modification of one or the other of the secondary sex characters, by the appearance of secondary sex characters of one sex in individuals of the opposite sex, as well as by the degeneration of ovary or testis or the transition of an ovary into a testis or of a testis into an ovary. In animals sex dimorphism is the characteristic thing, and one is familiar with such differences in sex as size, voice, stature, plumage, and the like.

Sex dimorphism in flowering plants, where the sexes are separate, is not very striking; secondary sex characters have been contrasted but little in such forms. Darwin (1889, page 11) cites the case of the Resteaceae of Australia and the Cape of Good Hope, forms which show extreme sex dimorphism. It is reported that often it is impossible to match the male with the female of the same species. Shull (1914) reports for *Lychnis dioica* L. a sex-limited character in the form of narrowness of leaf in the male of *Lychnis dioica angustifolia*. Cook (1914) reports on a case of sex inequality in hemp, where the male plants are smaller and shorter than the females. These male plants die much sooner than the females.

The female inflorescences of Mercurialis are borne in clusters in the axils of the leaves, while the male inflorescences are borne in interrupted spikes which surpass the leaves. This characteristic appearance of the inflorescences of the two sexes may be considered as the secondary sex character of the two sexes, in the sense that the manner in which the inflorescences are borne is characteristic for each sex. No doubt a closer examination of other dioecious forms will show differences in male and female pedicels, petals, and sepals, either by their presence or by their absence.

It is interesting to note that in sex intergradation in *Mercurialis annua* there is no transition of a secondary sex character of one sex into that of the other. Those females which tended towards maleness by producing many male flowers and many seeds did not take on the general growth characteristics of the male. The same holds true for the males that tended towards femaleness—they too still maintained their characteristic form of growth.

THE DOCTRINE OF VARYING POTENCIES IN GERM CELLS

Alternative inheritance of sex is the extreme of a series of intergraded variations. Hermaphrodites (with perfect flowers) and monoecious forms become dioecious not by the sudden development of heterozygosis in one sex and the separation of sex factors in the reduction division, but by the gradual development of sex purity (dioecism) through a long series of intergraded sex variants. The connecting links can all be found in the polygamous (mixed) species. If dioecism has arisen in this way, it is hardly likely that there is anything of the nature of fixed, invariable sex factors in the germ plasm. It is a matter of fluctuating tendencies. Male tends to produce male, female to produce female; sometimes one tendency is stronger, sometimes the other.

Strasburger (1910) attacks Correns' view that one sex is heterozygous for a sex determiner on phylogenetic grounds. The evolutionary trend has been to make the egg and sperm different. Phylogeny points to the egg's being female-producing and the male gamete's being male-producing. It is certainly an awkward assumption that one half the male gametes, for example, must carry female determiners. In an earlier work Strasburger (1909*a*) concluded that the egg of the dioecious phanerogam tends to produce females only, while in the production of the microspores of a tetrad by division of the pollen mother cell two of the spores will have a stronger male tendency than the other two. Those with the stronger male tendency (which is transmitted to their descendants, the male gametes) will dominate over the female tendency of the egg and thus males will be produced, while the weaker male tendency of the other two will be dominated by the stronger female tendency of the egg and females will result. Noll (1907), from his studies of dioecious plants, was led to this view that there are pollen grains of two strengths as regards the male-producing tendency.

While Strasburger's view explains the behavior of his selfed females and of his selfed males, and the sex of the progeny resulting from the fertilization of a female by a male, there is one difficulty that he overlooked. Assuming that the eggs are all of one kind, then the eggs produced on the male plants must all dominate over the weaker male gametes. Such however, is not the case. In the female that produces sporadic male flowers there is no reason, on Strasburger's assumption, why the male gametes should not be of two kinds. Selfed females produce only female offspring. That means that the male-producing tendencies transmitted by all the pollen grains of the tetrad are dominated by the female-producing tendency of the egg. On Strasburger's assumption there must now be at least three strengths of pollen grains, if not four: two kinds produced by the male, one of which is subordinate in its sex-determining tendency to the egg, and two kinds (on *a priori* grounds) produced by the sporadic male flowers on the female. Then, too, there are two kinds of eggs instead of one kind: the egg of the female plant which dominates over the weaker male-producing tendency of the pollen grains, and the egg produced upon the male plants, which is dominated by the male-producing tendencies of both kinds of pollen grains, and is thus weaker than the eggs borne on the female plant.

We reach here a conception, which the thus-far meager data on inheritance in dioecious and polygamo-dioecious forms seem to bear out, namely, that *there may be graded potencies in both the gametes, the egg as well as the male gamete, of such forms.* The work of Correns is especially significant. In his work on Satureja, Silene, and Plantago he brings out clearly that the more pronounced the sex of the individual the more marked will be its influence on the sex of its offspring. The normal appearance of sex intergrades (there are at least thirty degrees in *Plantago lanceolata* between pure female and hermaphrodite) is evidence in that direction. The behavior of the females of Mercurialis in my cultures is interesting in this respect. The original mother plant produced 66 seeds and 50 offspring. The offspring in turn produced seeds varying in number from 1 to 238. The original mother plant produced eggs of varying potencies as evidenced by the variation in male flower and seed production of the offspring. It is quite natural that the eggs should have varied in their ability to transmit the seed-producing qualities of the mother as in other qualities. Although the offspring tended to be like the mother in the sense that they were pure females or predominantly females, they varied in their ability to produce male flowers and hence seeds. The fertilized egg that produced a female that during its life history produced no male flowers or seeds is different, whether it be qualitatively or quantitatively, from the fertilized egg that produced a plant that produced many male flowers and seeds. One can conceive gradations in the power to produce male flowers and seeds, beginning with eggs with zero potentiality and running thence all the way to those with the potentiality of plant no. VII (Yampolsky, 1919), which produced 32 male flowers and 230 seeds.

The male cultures of *Mercurialis annua*, while they do not show the tendency toward intergradations as often as do the females, nevertheless bring out very clearly gradations in sex potency.

On the assumption that gametes vary or are graded in strength, the one-to-one ratio may be explained in dioecious forms, especially in dealing with mass populations. As has already been pointed out (*l.c.*), it is only when large numbers are considered that the one-to-one ratio appears. To be sure the law of chance comes into effect in such an explanation of sex ratios. The explanation of the one-to-one ratio may very well lie in the assumption that the gametes of the female have as much chance to dominate over the male gametes as the male gametes have to dominate over the female. That the gametes of one sex may in cases completely or almost completely dominate over those of the other sex is brought out in aberrant sex ratios. This advantage may, when large numbers of individuals are considered, be offset by a parallel condition resulting in the dominance of gametes of the other sex (Doncaster, 1913, 1916; Montgomery, 1908).

In Mercurialis, though the species is prevailingly dioecious, it is obvious that we must assume that the potentialities for the development of both sexes are present in practically all the individuals of the species. There is nowhere evidence that sex is determined in this plant by the presence or absence of a sex-determining factor. Those individuals which remain purely male or purely female throughout are not to be conceived as very different from those which produce a few flowers of the opposite sex. There is no evidence for the localization of the sex difference either in a special part of the plant or in a special part of the cell. The appearance of the sporadic flowers of the other sex may occur anywhere on the plant and at any stage of its development. Their occurrence is comparable to that of bud variation, and like the latter they show that the organism may contain latent potentialities as well as visibly expressed characters. Nor does the production of a few flowers of the other sex alter essentially the sex character of the plant as a whole. It is still prevailingly male or female and transmits its sex as such. It is highly probable that as a rule at least the pollen from sporadic male flowers on a female plant pollinates the nearest female flowers on the same branch. The seeds so produced, however, grow into female plants like the branch which bore them. It is sometimes questioned whether a plant with its potentialities of unlimited growth and with its succesive crops of reproductive organs is an individual in the sense that an animal is, with its more limited growth and definitely localized reproductive and other organs. The behavior of these prevailingly dioecious Mercurialis plants with reference to sex transmission certainly shows that they are unit individuals male or female in a very strict sense. But it is just as clear that, as noted above, the dioecious condition is only an extreme, a climax condition in the evolution of sex differentiation. As the data at the end of this paper show, the transition from the hermaphroditic and monoecious to the polygamo-dioecious and dioecious condition is going on at numerous and widely distributed points in the orders and families of seed plants.

Distribution of Sex Forms According to Engler and Gilg

- Monocotyledons
  - Order Pandanales
    - Pandanaceae: monoecious, dioecious, polygamo-dioecious
  - Order Helobiae
    - Potamogetonaceae: monoecious, dioecious, hermaphroditic
    - Naiadaceae: monoecious, dioecious, hermaphroditic
    - Scheuchzeriaceae: monoecious, dioecious, hermaphroditic
    - Alismataceae: monoecious, dioecious, hermaphroditic
    - Hydrocharitaceae: monoecious, dioecious, hermaphroditic
  - Order Triuridales
    - Triuridaceae: monoecious, dioecious, hermaphroditic
  - Order Glumiflorae
    - Gramineae: monoecious, dioecious, hermaphroditic
    - Cyperaceae: monoecious, dioecious, hermaphroditic
  - Order Principes
    - Palmae: monoecious, dioecious, hermaphroditic
  - Order Spathiflorae
    - Araceae: Monoecious, dioecious, hermaphroditic
  - Order Farinosae
    - Flagellariaceae: monoecious, hermaphroditic
    - Restionaceae: dioecious, hermaphroditic
    - Centrolepidaceae: monoecious, hermaphroditic
    - Eriocaulaceae: monoecious, dioecious, polygamous
    - Commelinaceae: monoecious, hermaphroditic
  - Order Liliiflorae
    - Liliaceae: mostly hermaphroditic, dioecious (Smilax, Britton)
    - Dioscoreaceae: monoecious, dioecious, hermaphroditic
  - Order Scitamineae
    - Musaceae: monoecious, hermaphroditic
    - Zingiberaceae: monoecious, hermaphroditic
    - Marantaceae: monoecious, hermaphroditic
  - Order Microspermae
    - Orchidaceae: mostly hermaphroditic
      - (Cataseteae): hermaphroditic, monoecious
- Dicotyledons
  - Order Piperales
    - Piperaceae: monoecious, hermaphroditic
    - Chloranthaceae: monoecious, hermaphroditic
  - Order Salicales
    - Salicaceae: monoecious, dioecious
  - Order Myricales
    - Myricaceae: monoecious, dioecious
  - Order Balanopsidales
    - Balanopsidaceae: dioecious
  - Order Leitneriales
    - Leitneriaceae: dioecious
  - Order Batidales
    - Batidaceae: dioecious
  - Order Julianiales
    - Julianiaceae: dioecious

- Order Fagales
    - Betulaceae: monoecious, rarely dioecious
    - Fagaceae: monoecious, rarely hermaphroditic
- Order Urticales
    - Ulmaceae: monoecious, dioecious, polygamous, hermaphroditic
    - Moraceae: monoecious, dioecious
    - Urticaceae: monoecious, dioecious, polygamous, hermaphroditic
- Order Proteales
    - Proteaceae: monoecious, hermaphroditic
- Order Santalales
    - Santalaceae: monoecious, dioecious, hermaphroditic
    - Loranthaceae: monoecious, dioecious, hermaphroditic
- Order Aristolochiales
    - Rafflesiaceae: monoecious, hermaphroditic
- Order Polygonales
    - Polygonaceae: monoecious, dioecious, polygamous, hermaphroditic
- Order Centrospermae
    - Chenopodiaceae: monoecious, dioecious, hermaphroditic
    - Amarantaceae: rarely monecious, dioecious, polygamous, hermaphroditic
    - Nyctaginaceae: monoecious, hermaphroditic
    - Phytolaccaceae: monoecious, polygamous, hermaphroditic
    - Caryophyllaceae: monoecious, dioecious
- Order Ranales
    - Ceratophyllaceae: monoecious, dioecious
    - Trochodendraceae: monoecious, hermaphroditic
    - Cercidiphyllaceae: dioecious
    - Ranunculaceae: dioecious, hermaphroditic
    - Lardizabalaceae: monoecious, hermaphroditic
    - Menispermaceae: dioecious
    - Magnoliaceae: monoecious, hermaphroditic
    - Monimiaceae: monoecious, hermaphroditic
    - Lauraceae: monoecious, dioecious, polygamous, hermaphroditic
    - Hernandiaceae: monoecious, hermaphroditic
- Order Rosales
    - Hydrostachyaceae: dioecious
    - Saxifragaceae: polygamo-dioecious, hermaphroditic
    - Hamamelidaceae: monoecious, polygamous, hermaphroditic
    - Rosaceae: polygamo-dioecious, hermaphroditic
    - Connaraceae: monoecious, hermaphroditic
    - Leguminosae: monoecious, dioecious, polygamo-dioecious, hermaphroditic
- Order Pandales
    - Pandaceae: dioecious
- Order Geraniales
    - Rutaceae: polygamo-dioecious, hermaphroditic
    - Simarubaceae: polygamous, dioecious, hermaphroditic
    - Burseraceae: dioecious, hermaphroditic
    - Dichapetalaceae: dioecious, hermaphroditic
    - Euphorbiaceae: monoecious, dioecious
    - Callitrichaceae: monoecious, hermaphroditic
- Order Sapindales
    - Buxaceae: monoecious, dioecious
    - Empetraceae: monoecious, dioecious, polygamous
    - Coriariaceae: monoecious, hermaphroditic

- Anacardiaceae: polygamo-dioecious, hermaphroditic
- Aquifoliaceae: dioecious, polygamo-dioecious
- Salvadoraceae: dioecious, hermaphroditic
- Icacinaceae: monoecious, hermaphroditic
- Aceraceae: dioecious, polygamous
- Hippocastanaceae: polygamous
- Sapindaceae: polygamo-dioecious
- Sabiaceae: polygamo-dioecious, hermaphroditic

Order Rhamnales
- Rhamnaceae: polygamous, hermaphroditic
- Vitaceae: polygamo-dioecious, hermaphroditic

Order Malvales
- Sterculariaceae: monoecious, hermaphroditic

Order Parietales
- Dilleniaceae: monoecious, hermaphroditic
- Guttiferae: monoecious, hermaphroditic
- Calophylloideae: monoecious, hermaphroditic
- Flacourtiaceae: monoecious, dioecious, hermaphroditic
- Stachyuraceae: polygamous, hermaphroditic
- Passifloraceae: monoecious, hermaphroditic
- Datiscaceae: monoecious, dioecious, hermaphroditic

Order Myrtiflorae
- Elaeagnaceae: monoecious, dioecious, polygamous, hermaphroditic
- Sonneratiaceae: monoecious, hermaphroditic
- Nyssaceae: monoecious, hermaphroditic
- Combretaceae: monoecious, hermaphroditic
- Halorrhagaceae: monoecious, dioecious, hermaphroditic
- Cynomoriaceae: monoecious, hermaphroditic

Order Umbelliflorae
- Araliaceae: monoecious, polygamous, hermaphroditic
- Umbelliferae: monoecious, polygamous, hermaphroditic
- Cornaceae: monoecious, hermaphroditic

Order Primulales
- Theophrastaceae: monoecious, hermaphroditic
- Mrysinaceae: monoecious, hermaphroditic

Order Ebenales
- Ebenaceae: dioecious, polygamous, hermaphroditic
- Styracaceae: hermaphroditic, rarely polygamo-dioecious (Britton)

Order Contortae
- Oleaceae: monoecious, dioecious, hermaphroditic
- Loganiaceae: monoecious, hermaphroditic
- Gentianaceae: monoecious, hermaphroditic

Order Plantaginales
- Plantaginaceae: monoecious, hermaphroditic

Order Rubiales
- Rubiaceae: rarely monoecious, hermaphroditic
- Valerianaceae: monoecious, dioecious, polygamo-dioecious, hermaphroditic

Order Cucurbitales
- Cucurbitaceae: monoecious, dioecious, hermaphroditic

Order Campanulatae
- Stylidiaceae: monoecious, hermaphroditic
- Calyceraceae: monoecious, hermaphroditic
- Compositae: monoecious, dioecious, polygamous, hermaphroditic

NUMBERS OF FAMILIES IN DIFFERENT ORDERS SHOWING THE VARIOUS TYPES OF SEX ARRANGEMENT.

Type I. Dioecious.
Type II. Dioecious, monoecious.
Type III. Dioecious, monoecious, hermaphroditic.
Type IV. Dioecious, hermaphroditic.
Type V. Dioecious, polygamous.
Type VI. Dioecious, polygamous, hermaphroditic.
Type VII. Dioecious, polygamous, monoecious.
Type VIII. Dioecious, polygamous, monoecious, hermaphroditic.
Type IX. Polygamous.
Type X. Polygamous, hermaphroditic.
Type XI. Polygamous, hermaphroditic, monoecious.
Type XII. Monoecious, hermaphroditic.

Order	Types											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Pandanales							1					
Helobiae			5									
Triuridales			1									
Glumiflorae			2									
Principes			1									
Spathiflorae			1									
Farinosae				1			1					3
Liliiflorae			1	1								
Scitamineae												3
Microspermae												1
Piperales												2
Salicales		1										
Myricales		1										
Balanopsidales	1											
Leitneriales	1											
Batidales	1											
Julianiales	1											
Fagales		1										1
Urticales	–	1	–	–	–	–	–	2				
Proteales												1
Santalales	–	–	2									
Aristolochiales												1
Polygonales								1				
Centrospermae	–	1	1				1	1	–	–	1	1
Ranales	2	1	–	1				1				5
Rosales	1							1	–	2	1	1
Pandales	1											
Geraniales	–	1	–	2	–	1				1		1
Sapindales	–	1	–	1	2	–	1		2	2		2
Rhamnales										2		
Malvales												1
Parietales			2							1		4
Myrtiflorae			1					1				4
Umbelliflorae			3									1
Primulales												2
Ebenales						1				1		
Contortae			1									2
Plantaginales												1
Rubiales								1				1
Cucurbitales			1									
Campanulatae								1				2
Total—Families	8	8	22	6	2	2	4	9	2	9	2	40
—Orders	7	8	13	5	1	2	4	8	1	6	2	21

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AN APPARATUS FOR AUTOMATICALLY CHANGING THE TEMPERATURE OF A CHAMBER

George F. Potter

It has been shown that the injury produced in certain plant tissues by freezing is influenced considerably by the rate at which the temperature falls during the freezing process.[1] In order to obtain a uniform and known rate of temperature fall for experiments of this sort, the writer has developed an apparatus in which the *rate of temperature change* is automatically controlled by clockwork. Any desired rise or fall of temperature can be obtained, the conditions desired for a ten-hour period being determined and recorded in advance. By repeating the experiment without altering the adjustments, different lots of tissues may be frozen under duplicate temperature conditions.

A longitudinal section of the freezing chamber, together with end, top, and side views of the controlling mechanism are shown in Plate I. Details of certain portions of the apparatus, as indicated by lettering analogous to that used in Plate I, are shown in both front and side view in Plate II. The freezer proper consists of three cylindrical galvanized iron cans, placed one within the other, riveted together, and packed in a box of sawdust for insulation. The freezing mixture is placed in the space between the two outside cans (*a*, Plate I). The innermost can (*c*, Plate I) is the freezing chamber. The intervening space (*b*, Plate I) is used as an air space to prevent too rapid cooling unless temperatures lower than—10° C. are desired, in which case it is filled with an additional quantity of ice and salt. The two outside chambers (*a* and *b*, Plate I) are fitted with pipes and stopcocks for drawing off the brine. The opening through these must be straight to facilitate removing obstructions.

The freezing chamber is fitted with a tight galvanized iron cover, to the center of which a short section of iron pipe is attached firmly by means of an iron collar. All thermometers, recording and control apparatus are introduced through this opening. A steel T-bar is securely clamped to the iron pipe (section *BB*, Plate II) and extends from slightly above the top of the pipe to within a few inches of the bottom of the chamber. This bar serves as a support for both fan shaft and thermostat, and by means of clamps holds interchangeable racks to which the experimental materials are attached. All three cans are covered by a single galvanized iron cover of circular shape insulated with cork board. The iron pipe from the inner chamber passes through an opening in the exact center of the insulated cover, which is therefore free to revolve about the pipe. Ice and salt may be placed in any part of the two outer spaces (*a* and *b*, Plate I), through an opening near the outer edge of this cover.

---

[1] Chandler, W. H. Killing of plant tissue by low temperature. Mo. Agric. Exp. Sta. Research Bull. **8**: 199–205. 1913.

In such a chamber it is always difficult to keep the temperature uniform from top to bottom. To accomplish this as nearly as possible, a false wall or tin cylinder about two inches smaller in diameter than the freezing chamber, is introduced and held in place with wooden blocks (longitudinal section of freezer, Plate I). The cold wall of the chamber outside and the heating coil within cause the air to circulate upward inside the false wall and downward outside it. In addition, the circulation is forced by a fan at the bottom of the chamber. When one is freezing parts of plants that do not obstruct the passage of air, thermometer readings indicate that there is less than 0.1° C. difference in temperature over a vertical distance of eight inches.

The heating coil is wound with about 30 feet of no. 30 "Chromel C" resistance wire, having a total resistance of about 200 ohms. As a rule a large low-resistance lamp, or two 40-watt lamps in parallel, are placed in series with the coil to reduce the amount of heat given off and to act as a pilot light. On a 110-volt A. C. lighting circuit about one half ampere or less of current is used. The lamp can be switched out of the circuit if more heat is needed, as for instance when a large quantity of freezing mixture has just been added. An ordinary "Dim-a-lite" or "Hylo" connection is also placed in the circuit and can be used to reduce the amount of heat given off by the coil, as for instance when the ice is nearly exhausted. These adjustments are frequently convenient, although not necessary for the operation of the machine.

A mercury thermostat (*C*, Plate II) controls the heating coil by means of a telegraphic relay operating on current from two dry cells. When the machine is operated continuously it is necessary to have two batteries of two cells each. One battery may then recuperate while the other is in use. The connection is conveniently alternated by means of a double throw switch. The thermostat consists of a piece of capillary tubing sealed to a bulb containing mercury. An enlargement at the top of the capillary holds the excess mercury at temperatures above the working range. The thermostat used by the writer has a bulb about 1 cm. in diameter by 13 cm. in length and contains sufficient mercury to cause the mercury in the capillary to rise or fall about 2 mm. for each degree Centigrade change of temperature. Electric connection is made between the mercury in the bulb and that in a side arm by means of a platinum wire sealed in the side of the bulb (*C*, Plate II). A platinum wire guided to the exact center of the capillary by a small glass rod makes contact at the top of the mercury column (*CC*, Plate II). The guide is indispensable for accurate results because it makes an appreciable difference in temperature whether contact is made at the center or at the edge of the convex meniscus. An insulated wire is run from the battery to the side arm. The metal parts of the apparatus acting as a ground wire carry the current to the contact in the capillary. A condenser is used to prevent sparks at the point of contact with the mercury, and to eliminate the arc completely it is necessary to short circuit the terminals of the condenser through a resistance of about 700 ohms. A fifteen-watt Mazda bulb is used for this purpose.

The point at which the platinum wire touches the mercury in the capillary tube controls the temperature at which the heating coil is brought into use, and hence controls the temperature of the chamber. The contact wire is attached to a plunger, which moves vertically in guides fastened to the T-bar above the thermostat (longitudinal section, Plate I, and *B* and *C*, Plate II). The mechanism which actuates this plunger is similar in principle to a recording thermometer (*E*, *F*, and *G*, Plate I). The hands were removed from a "Big Ben" alarm clock and a shaft, bearing a drum three inches in diameter and four inches long, was soldered to the hour-hand shaft. A time-temperature chart is attached to the drum, degrees being marked by equal spaces along the axis of the cylinder, and hours by twelve equal spaces about its circumference. The distance to be laid off for each degree depends on the sensitivity of the thermostat and on the relative lengths of the two levers which will be referred to as the "long arm" and "short arm" of the "revolving shaft." The time-temperature curve is constructed with a flexible lead bar, fastened at each end by a clamp (*e*) sliding in a groove which runs lengthwise of the drum (*F* and *G*, Plate I). Below the drum and at right angles to it there is a revolving shaft (*E*, Plate I). At the clockwork end of this shaft a long arm, somewhat similar to that which carries the recording pen of the thermograph, extends upward and engages the lead bar with a small connecting pin (*E* and *G*, Plate I). At the other end of the shaft, which is pivoted in a bearing at the top of the T-bar leading down into the freezing chamber, there is a short arm which is joined by means of a connecting rod to the plunger carrying the platinum contact wire of the thermostat (*A* and *B*, Plate II). The weight of the connecting rod and plunger acting on the short arm as a lever tends to revolve the shaft and thus keeps the connecting pin of the long arm in contact with the edge of the lead bar. As the drum revolves, a movement of the long arm is permitted in accordance with the curve traced by the lead bar, and a proportionate movement is transmitted to the plunger and contact point of the thermostat through the turning of the shaft and through the resulting movement of the short arm and connecting rod. The short arm is attached to the shaft in such a way that it forms a right angle with the connecting rod when the long arm is at right angles to the axis of the drum. The vertical movement of the plunger is therefore always exactly proportionate to the horizontal distance which the long arm moves along the axis of the drum, although the amount that the shaft revolves for each degree of temperature is greater as the arm approaches either end of the drum.

Regulation of the apparatus is accomplished by means of an adjusting screw at the top of the connecting rod (*A*, Plate II). The temperature within the chamber is read with a thermometer. The drum is revolved until the long arm indicates the same temperature on the chart. The screw is then turned until the platinum point just makes a contact with the mercury in the capillary. In making this adjustment it is necessary to allow for some "lag" in the thermostat if temperatures are changing rapidly in the chamber and if the thermometer used is a sensitive one. As a rule the apparatus is held about fifteen minutes at the adjusting temperature. In operation, the "lag," using the large thermostat mentioned above, did not cause variations in temperature of more than 0.25° C. when the temperature of the chamber was changing at a rate of 16° C. per hour.

The connecting rod is so designed that it may be lifted up and detached from its support on the short arm of the revolving shaft. The base of the clock mechanism is fastened to a slotted support by a screw clamp. By detaching the connecting rod and loosening the clamp, shaft and clock can be removed. The insulated wire leading to the thermostat is then disconnected from its binding post and the covers of the freezing chamber may be removed.

The use of this machine has enabled the writer to perform freezing experiments under conditions controlled more accurately than has been possible even with the closest personal attention when using a hand-controlled freezer. The range and accuracy of regulation of temperature depend almost entirely upon the thermostat. The instrument used by the writer works through a range of 10° C., and the variations between the temperature indicated on the chart and that observed in the chamber usually are not greater than 0.1° C., although sometimes variations of 0.25° C. are observed. A less sensitive thermostat capable of working through a correspondingly greater range of 40° C. showed maximum variations of about 0.5° C. However, if particles of mercury become separated from the top of the column in the capillary tube, the operation of the machine becomes unreliable and usually the temperature of the chamber becomes too high. To avoid this the capillary must be cleaned of any oil or dirt about once a week, or sometimes oftener if foreign materials chance to enter. *It is also necessary to keep the glass guiding rod of the contact point (CC, Plate II) above the surface of the mercury because if immersed it breaks the column,* carrying part of the mercury up above the rod. It is possible that this difficulty could be eliminated by the use of some other type of thermostat, but the writer has not been able to find one which has the same sensitivity, and at the same time the ability to return accurately to the original starting point after going through wide temperature variations. The last mentioned characteristic is absolutely essential for the operation of a machine of this sort. Even with these limitations the machine enables the experimenter to do much more accurately controlled work than would be possible without it. The writer's record book shows a total of 74 experiments performed in February and March, 1919. Seven of these were of at least 48 hours duration, about half of the remainder 12 hours, and none less than 6 hours. Most of the time one short experiment was carried on during the day, and one of twelve or more hours' duration was run during the night. Out of the total, seven were discarded because the final temperature was more than 0.25° C. from that desired.

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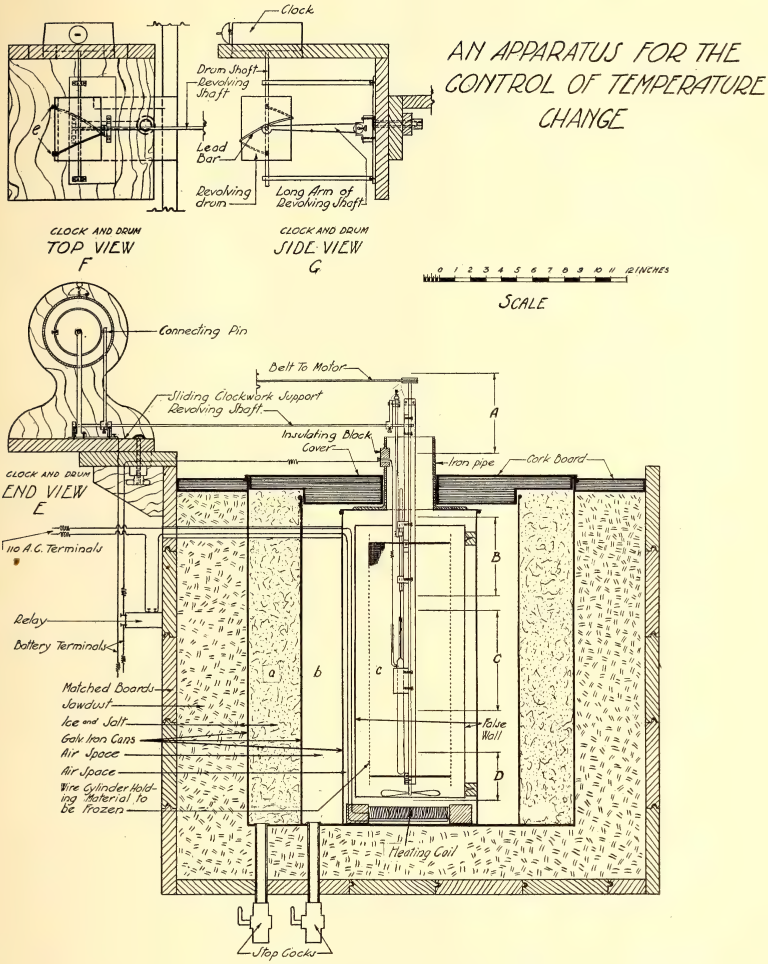
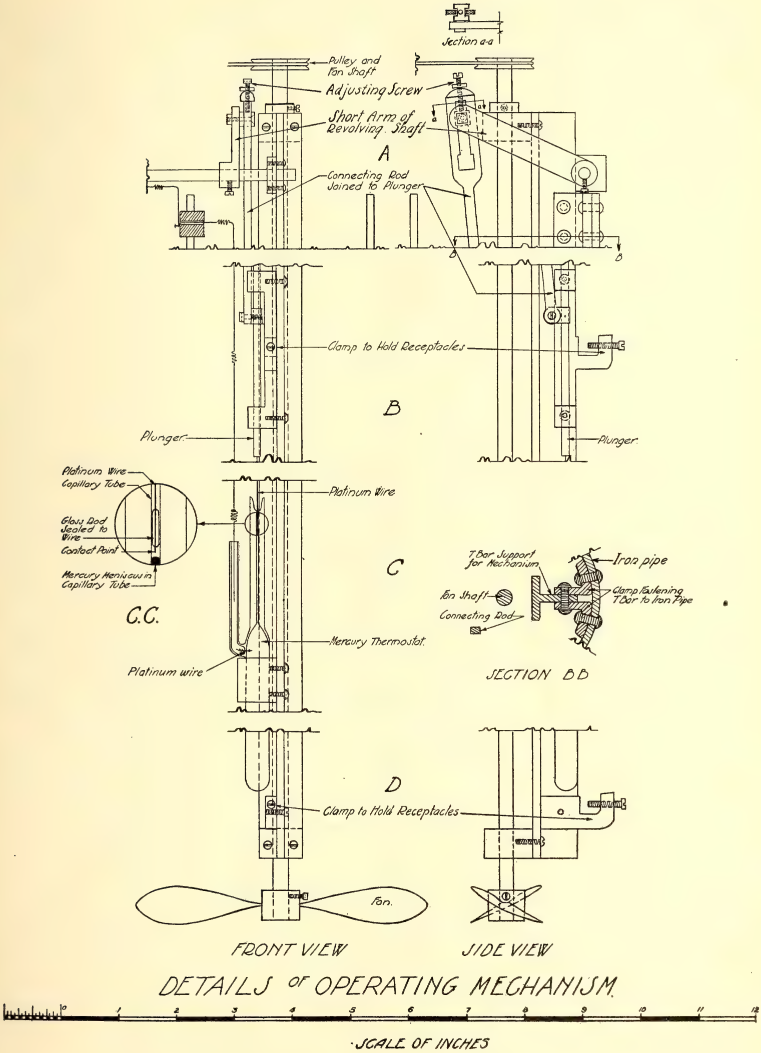

AN APPARATUS FOR THE CONTROL OF TEMPERATURE CHANGE
Clock
Drum Shaft
Revolving Shaft
Lead Bar
Revolving drum
Long Arm of Revolving Shaft
e
CLOCK AND DRUM TOP VIEW F
CLOCK AND DRUM SIDE VIEW G
0 1 2 3 4 5 6 7 8 9 10 11 12 INCHES
SCALE
Connecting Pin
Belt To Motor
Sliding Clockwork Support
Revolving Shaft
Insulating Block
Cover
Iron pipe
Cork Board
A
B
C
D
CLOCK AND DRUM END VIEW E
110 A.C. Terminals
Relay
Battery Terminals
Matched Boards
Sawdust
Ice and Salt
Galv. Iron Cans
Air Space
Air Space
Wire Cylinder Holding Material to be Frozen
a
b
c
False Wall
Heating Coil
Stop Cocks
LONGITUDINAL SECTION

POTTER: APPARATUS FOR CONTROL OF TEMPERATURE.


Section a-a
Pulley and Fan Shaft
Adjusting Screw
Short Arm of Revolving Shaft
A
Connecting Rod Joined to Plunger
a
a
B
B
Clamp to Hold Receptacles
B
Plunger
Plunger
Platinum Wire
Capillary Tube
Glass Rod Sealed to Wire
Contact Point
Mercury Meniscus in Capillary Tube
C.C.
Platinum Wire
C
Mercury Thermostat
Platinum wire
T Bar Support for Mechanism
Iron pipe
Fan Shaft
Clamp Fastening T Bar to Iron Pipe
Connecting Rod
SECTION BB
D
Clamp to Hold Receptacles
Fan.
FRONT VIEW
SIDE VIEW
DETAILS OF OPERATING MECHANISM
0
1
2
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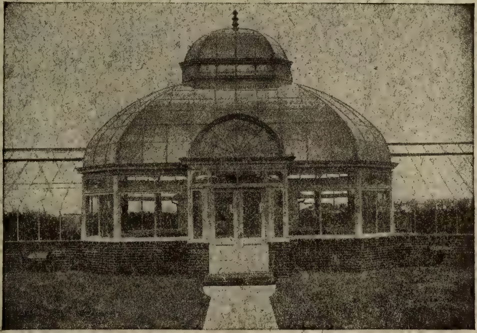
11
12
SCALE OF INCHES

Potter: Apparatus for Control of Temperature.



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EFFECT ON CHESTNUTS OF SUBSTANCES INJECTED INTO THEIR TRUNKS

CAROLINE RUMBOLD

These observations are based on tree injections made during the summers of 1912, 1913, 1914, and 1915.[1] All the trees were orchard chestnuts: Paragon scions grafted on *Castanea dentata.*

TREES INJECTED WITH DYES

Some evidence concerning the path of the injected solution in the tree was obtained from stains. In 1912, 0.01 percent solutions of eosin, methyl green, and Congo red were injected into the trees. Although the holes were cut into the heartwood, the stains were found in the vessels of the youngest, or last annual ring; from here the stain spread slightly through the surrounding tissue. It was found that the stain descended as well as ascended in these vessels. In the roots the path of the stain was not followed to the root tips; it was found in the ring of vessels. In the small branches and twigs of the tree the stain often encircled the wood, whereas in the trunk lower down it had been seen in patches only in the last year's ring of vessels. The dyes varied in their effect on the trees. The eosin stain passed into the leaves of the injected trees and was toxic in the dilution used. Methyl green was not a good stain in that the color showed a tendency to fade. Illustrations of sections of these injected trees are in the report of the physiologist of the Pennsylvania Chestnut Blight Commission for the year 1912.[2]

In 1913, the non-toxic dyes, methylene blue, Congo red, and trypan blue, in 1/40 percent solution were injected for twenty days into six small trees, the injections starting April 16. At first the solutions flowed rapidly into the trees, especially the methylene blue, then more slowly, and had practically ceased going in when the injections were stopped. The trees were cut down in October. Two of these trees were infected with the

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[1] Rumbold, C. The injection of chemicals into chestnut trees. Amer. Journ. Bot. 7: 1–20. 1920.

[2] Rumbold, C. Report of the physiologist. Report of the Pennsylvania Chestnut Tree Blight Commission, Harrisburg, July 1 to December 31, 1912, pp. 45–47, figs. 43–47. 1913.

[The Journal for January (7: 1–44) was issued February 25, 1920.]

chestnut blight fungus; one had just been girdled at the base of the tree. The stain extended into the branches and roots, sometimes but part way, sometimes to the beginning of the year's growth. In no case did it extend beyond the beginning of the new year's growth of twigs and roots. At the point of injection it had penetrated three annual rings of wood and was found in the bark. However it was soon confined to the last annual ring in the neighborhood of the vessels. The girdled tree showed the stain passing through the diseased area by way of the vessels and tracheids; traces of it were found in the bark where disintegration had just begun. It will be noticed that the results of injection in 1912 and 1913 differed. No experiments were undertaken to discover the cause of this difference.

As stated before, most of the dye passed through the xylem elements of the last formed annual ring. At first it entered the large spring vessels, which appeared, however, not always to be the carriers. The summer tracheae or vessels, which in cross sections of Paragon chestnut form characteristic flame-like lines of pores diverging from the spring vessels, were deeply stained, and so were the tracheids. The trees injected with methylene blue showed ragged tissues and holes in the neighborhood of the spring vessels. There were traces of dye found in some of the vessels of the new growth of wood near the point of injection, but generally this new growth was unstained.

A microscopic examination of the xylem cells showed that the dye was retained by the walls of the vessels and tracheids through which it passed.

These phenomena (the decreasing size of the injected area and the gradual dilution of the solution as the distance from the point of injection increased) were observed when solutions of salts were injected into growing trees. Though the paths could not be followed as easily as when dyes were used, they could be traced, often, by a formation of abnormal bark tissue which disappeared as the distance from the point of injection increased. When a "killing" solution was injected the path was marked on the trunk by vertical strips of dead tissues. Those twigs and branches whose vascular system entered this path were killed; often but one side of a branch was affected. All stages of reaction to an injection could be seen in a tree: dead tissue at the point of injection in the trunk, dead or falling leaves on the branches nearest the injection hole, spotted leaves on branches higher up the tree, and no signs of injection visible in the top of the tree.

Time of Injection and Dilution of Solution

The way in which a tree was affected depended both on the time of the injection and on the dilution of the chemical solution. Concentrated solutions acted more quickly than dilute ones and generally were injurious.[3]

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[3] "Experiments on the P_H or true acidity values of normal and cankered chestnut bark adjacent to the cambiam layer show that healthy chestnut bark has a P_H of about 4.8,

Alkali Metals

In September the effects of an injection of lithium carbonate 1/20 G.M. were visible in the leaves three days after the injection started. The leaves nearest the point of injection were first affected, those furthest distant last. When the dilution was 1/200 G.M. or weaker, the leaves first affected were at the ends of the branches; in August they were those nearest the burs. Trees injected in late summer with a 1/20 G.M. dilution produced normal leaves in the following spring at the normal time, but after the leaves were full grown they gradually showed the characteristic lithium curling and spotting (Pl. I, fig. A), although no more injections had been made in the trees. The other lithium salts acted in the same manner.

What happened in the case of the lithium salts, happened to some extent with the other alkali metals (ammonium compounds excepted). Sodium solutions 1/20 G.M. in strength produced effects like those of the lithium salts; but when diluted to 1/200 G.M. they did not blotch the leaves or affect the young bark, so that the presence of unusual quantities of sodium in the branches could not be vouched for. Potassium salts behaved like the sodium. It was observed in October that the sodium-, potassium-, and ammonium-injected trees (1/100 G.M. and 1/200 G.M.) generally lost the leaves on the ends of the branches near the burs first, and that this leaf fall was previous to the fall on the water-injected trees. The ammonium salts: chloride 1/200 G.M., carbonate 1/100 G.M., and hydroxide 1/100 G.M., also appeared to affect the leaves on the ends of the branches, causing them to drop; the sulphate 1/200 and 1/500 G.M. blotched the leaves. The normal growth of the trees was not seriously affected by the injection of the alkali metals.

Heavy Metals

Of the heavy metals injected, potassium chromate and bichromate and copper sulphate and chloride showed their effects most quickly. The chromates were more toxic and spread through the tree more quickly than the copper salts; the bichromate was more poisonous than the chromate.

The leaves of the tree injected with the chromate solutions became affected in 48 hours. The veins of the leaves browned first, then the leaves curled upward, dried, and dropped off. New leaves formed, but they in turn fell. The dilution 1/10000 G.M. of potassium chromate behaved like the 1/20 G.M. copper salts. With the exception of those trees injected with this latter dilution, the trees of this series were almost bare in August.

whereas samples of cankered bark have shown P_H values as low as 3.24. A P_H of 3.24 represents an acidity about 23.7 times that of 4.8. When N/100 and N/1000 alkalies are injected into the tree the acidity automatically increases very slightly as a kind of immunity to offset the effect of the alkali. When larger quantities of N/10 alkali are injected the sap becomes decidedly alkaline and the tree dies. The details of these investigations will appear later in the Journal of Agricultural Research." (C. Rumbold, M. R. Meacham and S. F. Acree.)

Ten days after being treated with potassium bichromate 1/1000 G.M., the back of the tree cracked along the edges of the path of the solution. In July and August, *Penicillium* sp. grew luxuriantly in these cracks and in the points of injection. The following year all the trees injected with the chromate solutions were dead.

The day after an injection of copper sulphate 1/20 G.M. was started, the leaves began to turn brown, those nearest the point of injection first. Copper chloride 1/20 G.M., zinc chloride 1/20 G.M., and barium chloride 1/20 G.M. acted almost as quickly. All of these were "killing" solutions. As previously stated, the paths these solutions took up and down the tree could be followed by the visible killing of the tissues. The region they passed through was a narrow one, but little wider than the hole made for the injection. Those twigs and branches whose fibers entered this path showed dead leaves. The leaves were the first to show the effects those nearest the point of injection browning first. A smell of decaying plant tissue became noticeable (in the case of Cu_2SO_4 1/20 G.M. in 10 hours, during which time 1 liter had been injected), which sometimes continued for one and two days. The dying leaves did not become crisp until some time after they had browned, in one case not until four days after browning. Environmental conditions probably influenced this phenomenon. Eventually all the leaves on the trees died, and soon those on the parts of the trees not included in the paths of the solutions fell off. The leaves dropped as they would in the autumn. The denuded branches quickly produced new leaves, so that trees injected in August had full-sized green leaves in December. The dead leaves still hung on the injected branches, rendering them conspicuous. The following spring these trees leafed, and produced fruit like the surrounding trees. The branches which had been injected were dead. The effect on the other parts was as though the trees had been severely pruned.

Colloidal Metals

The solutions of the heavy metals proved detrimental to the normal growth of the trees. The colloidal metallic solutions were exceptions. Examination of the injected trees indicated that most of the injected colloids stayed in the trunks near the place of injection.

Carbon Compounds

Two of the carbon compound solutions proved very toxic when injected. Four-tenths percent formaldehyde[4] affected the trees much as did the stronger concentrations of the copper solutions, but more severely for the reason that formaldehyde made broader paths when passing up the trunks. The trees above the point of injection were dead the following spring, but produced suckers from the base of the tree and from buds near the base.

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[4] Schering.

Meta-cresol 1/1000 G.M. killed the tissues as it passed up and down the tree. The midribs and veins of the leaves browned and exuded a smell of creosote. Finally they turned black and shriveled, hanging to the twigs as though scorched by fire. Along the sides of the path of the solution callus formed. The bark peeled from the injected area and exposed the wood. Outside this path the tree was unaffected (Pl. I, figs. *C* and *D*).

The dilutions of the carbon compounds injected, with the two above noted exceptions, did not, apparently, seriously affect the normal growth of the trees, though some of them caused blotching of the leaves.

Extracts

Canker extract killed the trees. Water extract of healthy bark did not affect them.

Water

Water injected into trees for three succeeding years apparently in no way modified their growth.

Discoloration of Leaves Due to Injection

Some of the solutions injected affected the leaves in so marked a manner that one could tell from the type of blotching what base had been introduced.

Lithium produced the most characteristic blotches of all the substances. These blotches appeared irrespective of whether a carbonate, hydroxide, chloride, nitrate, or sulphate was introduced. Usually the tip and the edge of the leaf between the veins turned a reddish brown color, giving the leaf a scalloped appearance (Pl. III, fig. *A*). Sometimes, however, these spots appeared in the parenchyma in the middle of the leaf. A dark line separated the green from the brown area. The leaf curled upward. As more lithium accumulated, the discolored area advanced toward the midrib. The base of the leaf was the last to turn brown.

Sodium carbonate 1/20 G.M. killed the leaf parenchyma in somewhat large irregular areas, which sometimes were in the central part of the leaf extending across veins and leaving the leaf edges green. The division between green and brown areas was sharply defined. Dilute solutions of sodium salts did not blotch the leaves. The potassium salts in the dilutions used in the injections did not blotch the leaves.

Ammonium compounds did not brown the leaves, but ammonium sulphate 1/200 G. M. and 1/500 G.M. caused a wrinkling or frilling of the leaf edges. This frilled area became translucent and later brittle, and the network of small veins showed prominently. Occasionally these wrinkled areas looked bleached, and were surrounded by a dark green band.

The colloidal metals did not visibly affect the leaves.

Concentrated heavy metal solutions produced three varieties of discolored leaves; one, a browning of the midrib and veins, which gave the leaves a finely checked appearance. The parenchyma browned last. The leaves then became dry and curled upward. There was another kind of discoloration characteristic of these solutions which appeared on leaves distant from the point of injection, or at a point where the solutions injected were diluted. Irregular brown spots appeared on the edges of the leaves which spread gradually toward the green petiole. The line of demarcation between brown and green areas was sharply defined. Such leaves were found on all trees injected with heavy metals. This effect in turn was quite different from that produced on leaves in the uninjected parts of trees treated with concentrated solutions, where a gradual bleaching appeared.

The manner in which formaldehyde 4/10 percent and meta-cresol 1/1000 G.M. affected leaves has been described. While meta-cresol proved so toxic, para-cresol 1/1000 G.M. produced no apparent effect on the leaves.

Those carbon-compound-injected trees which had discolored leaves showed two variations of discoloration. Para-nitro-phenol 1/500 G.M. browned the midribs and veins of leaves near the point of injection. Those leaves gathered from more distant parts showed light brown blotches on the edges which gradually advanced toward the base of the leaf. (The leaves, as far as appearance was concerned, could have been taken from a tree injected with $HgCl_2$ 1/1000 G.M.) Trees injected with the 1/1000 G.M. solution of para-nitro-phenol also showed these two varieties of discolored leaves. Ortho-nitro-phenol 1/1000 G.M. produced effects on leaves resembling those on ammonium-sulphate-injected trees, the leaves having translucent, brittle, frilled edges.

Picric acid 1/1000 G.M. caused the appearance of blotched and frilled leaves; citric acid 1/50 G.M., of blotched leaves; citric acid 1/500 G.M., of blotched and frilled leaves; acetic acid 1/500 G.M., of blotched leaves; formic acid 1/1000 G.M., of blotched leaves; salicylic acid 1/5000 G.M., of blotched and frilled leaves; pyrogallic acid 1/1000 G.M., of blotched leaves, the entire leaf finally turning a bright yellow and dropping off, as well as of frilled leaves; phloroglucine 1/1000 G.M., of frilled leaves; pyrocatechin 1/1000 G.M., of frilled leaves.

A possible explanation for these three variations in the discoloration of the leaves on an injected tree is that the leaves became impregnated in the course of the solution's spread with varying dilutions of the injected substance, those at a distance being impregnated with a much more dilute solution than those near the place of injection. The more concentrated solutions killed the tissues as they passed, thus browning the midribs and veins of the leaves, leaving the parenchyma green. When sufficiently dilute they flowed into the leaves without apparent harm, but gradually accumulated through transpiration in the parenchyma cells until a poisonous effect was produced. The third variation, that in which the leaf edges wrinkled or frilled, may be the effect not of the substance originally injected, but of by-products resulting from injuries caused by its presence in the lower parts of the tree. These three variations did not appear on every injected tree; sometimes there was but one kind, sometimes there were but two kinds of discolored leaves.

EFFECT ON TRUNKS

The holes made for the injections usually were filled with grafting wax after the removal of the injection tubes. A callus growing from both sides of the wound gradually closed it, leaving a small slit hardly noticeable on the tree. Sometimes this callus forced the wax from the hole, sometimes completely closed it in. It was found on examining felled trees that callus might cover the injection wound while an air space extended from the point of injection up and down the tree trunk between the outer bark and the wood (Pl. III, fig. *B*). This hole or tunnel was caused by the failure of the new annual ring to grow at that point, the cambium layer having been killed by the injected fluid. Such holes, first noticed in trees injected with lithium salts, were found to be a somewhat usual result of injection. Trees treated with meta-cresol 1/1000 G.M., formaldehyde 0.4 percent, potassium bichromate 1/1000 G.M., or mercuric chloride 1/1000 G.M., showed these holes in marked degree in that the bark cracked and peeled away from the treated area. The lithium-injected tree, first noticed, had been injected in the late fall and the injection wound had been left uncovered. In the spring of the following year, this hole was found filled with water below the point of injection. It was uncovered by cutting away the bark. There was no chestnut blight infection found and callus had formed along its sides. It extended from a point at the base of the tree about three feet below the point of injection to a point somewhat less than three feet above the hole. It was thought that some of these holes might be formed beneath the bark by the eroding effect of the extraordinary amount of foreign fluid passing through a narrow channel rather than by the toxic character of the fluid. Trees injected with methylene blue showed this disintegration in a less marked form. Primarily, the nature of the solution injected determined the formation of these holes and their size, for an examination of the injected trees showed that weak acids, water, and extracts did not produce such holes. A tree into which para-nitro-phenol 1/1000 G.M. had been injected, and in which one injection ran for more than five weeks, showed short and rather narrow holes. The colloidal metals produced no holes, nor was there an abnormal growth of tissue.

The "killing" solutions produced no stimulation of growth further than the callus which cut off the dead tissue from the living. Solutions more dilute did not kill the tissue outright, but caused the formation of wound tissue in the growing annual ring and bark.

Effect on Fruits

All the injected trees with the exception of those treated with the concentrated solutions of the heavy metals and formaldehyde produced a normal appearing crop of nuts.

There was no sign of a stimulation of the trees by the substances injected further than that the nuts growing on trees treated with the alkali metals in general appeared somewhat larger and glossier than those on trees injected with water or carbon compounds. Lithium was found in the nuts gathered from the trees injected with the lithium salts. The contents of the nuts gathered from the other injected trees were not tested further than by a superficial feeding experiment with white rats[5] to test their possible poisonous effect. In view of the fact that lithium was found in the nuts, it seemed possible that some of the other injected substances had found their way into the fruits. The amount of poison in them must have been extremely small since they did not appear to injure the rats' health. Another indication of this lack of toxicity was a count made of the wormy nuts gathered from treated and untreated trees in the orchard. This count showed the percentage of wormy nuts to be the same for both classes of trees.

It seems possible, judging from the varying results of the injections made in the spring and fall, that the amount of injected substance which finally reaches the nuts can be influenced by the time of injection. The late summer injections quickly affected the chestnut fruits, as shown by the spotting of the burs and neighboring leaves when injected with lithium.

For the sake of brevity the substances injected are arranged as carefully as possible in groups according to the effect they produced on the trees during the summers of experimentation. Very often the trees did not respond in the same degree to injections of the same chemical so that it was difficult to judge its general effect, and possibly some of these dilutions of chemicals could be put in two groups.

No apparent effect on trees
Water
Water extract of healthy chestnut tree bark
Congo red 1/40 percent
Trypan blue 1/40 percent
Colloidal cuprous hydroxide 1/3300 G.M.
Colloidal metallic silver 1/6400 G.M.
Methyl alcohol 1/100 G.M.
Acetic acid 1/3000 G.M.
Formic acid 1/6000 G.M.
Lactic acid 1/1000, 1/2000 G.M.
Anilin sulphate 1/1000 G.M.
Sodium carbolate 1/1000 G.M.
Phenol Sodique, 1 cc. to 1000 cc. H_2O
Para-nitro-phenol 1/10000 G.M.
Para-cresol 1/1000 G.M.
Thymol 1/3000 G.M.
Oil of bitter almonds 1/10000 G.M.

[5] Chestnuts were gathered from each injected tree and kept separate in labeled paper bags. Twelve rats were fed regularly with the chestnuts. A day of chestnut feeding (the nuts for the day being those gathered from trees injected with a particular chemical) alternated with one of bread, milk, and grain.

The ammonium compounds 1/500 G.M.

Apparently a slight stimulant

The weaker dilutions of the alkali metals
Para-nitro-phenol 1/1000 G.M.
Picric acid 1/10000 G.M.

Slightly detrimental (blotched leaves, death of cambium near the point of injection)

Para-nitro-phenol 1/500 G.M.
Ortho-nitro-phenol 1/1000 G.M.
Picric acid 1/500, 1/1000 G.M.
Pyrocatechin 1/1000 G.M.
Pyrogallic acid 1/1000, 1/500 G.M.
Phloroglucine 1/1000 G.M.
Benzoic acid 1/500 G.M.
Phenol 1/1000, 1/500 G.M.
Copper sulphate 1/100 G.M.
Lithium salts 1/100 G.M.
Ammonium compounds 1/100 G.M.
Sodium chloride 1/100 G.M.
Eosin 1/40 percent
Methylene blue 1/40 percent
Acetic acid 1/1000 G.M.
Formic acid 1/1000 G.M.
Citric acid 1/50, 1/500 G.M.

Detrimental (death of injected part of tree or of whole tree)

Copper sulphate 1/20 G.M.
Copper chloride 1/20 G.M.
Zinc carbonate 1/20 G.M.
Mercuric chloride 1/1000 G.M.
Potassium chromate 1/1000, 1/10000 G.M.
Potassium bichromate 1/1000, 1/10000 G.M.
Barium chloride 1/20 G.M.
Alkali metals 1/20 G.M. (NaCl 1/50 G.M.)
Formalin 0.4 percent
Acetic acid 1/100 G.M.
Formic acid 1/100 G.M.
Lactic acid 1/100 G.M.
Anilin sulphate 1/100 G.M.
Meta-cresol 1/1000 G.M.
Benzoic acid 1/500 G.M.
Salicylic acid 1/100 G.M.
Water extract of chestnut blight canker

SUMMARY

For four years observations have been made on the effect of chemical solutions injected into the trunks of chestnut trees.

1. Usually it was found that the visible effect of a solution on a tree varied with the distance from the point of injection.

2. The effect varied with the dilution of the solution and the month in which the injection was made.

3. In general the effect of the injection of the alkali metals was not detrimental to the trees; injection of heavy metals was detrimental; colloidal metals were not detrimental; organic compounds were not detrimental; water extract of chestnut blight canker was detrimental, healthy bark extract was not.

4. Many of the bases produced characteristic discoloration of the leaves.

5. Lithium was found in the nuts gathered from lithium-injected trees. The nuts gathered from the remaining trees were not tested sufficiently to show positively whether or not they contained any of the injected chemicals.

THE EFFECT OF THE INJECTED CHEMICALS ON THE FUNGUS ENDOTHIA PARASITICA

The results of the injections on the growth of the chestnut blight canker on the chestnut tree have been so uncertain and varied that, were it not for the fact that the work must stop for the present, no results would be mentioned.

It seems best to give a history of the results as they presented themselves.

The first indication of an effect from the injected chemicals on the fungus was in the summer of 1913. The trees which had been injected in 1912 had been inoculated with the chestnut blight fungus in the fall. The fungous growth from these inoculations on those trees injected with alkali metals had an abnormal appearance. However, the fungus continued to grow and eventually killed the trees. This abnormal appearance of the fungus together with the fact that the alkali-injected trees had, as a whole, a thrifty look led to the decision to put more emphasis upon the injection of the alkali metals.

In 1914, measurements were made of the cankers caused by the inoculations of 1913. These showed that the cankers on the control trees averaged the same size as those on the alkali-injected trees. The measurements of the cankers on the other injected trees gave confused results. As a whole the injected trees had larger cankers than the uninjected.

In 1915, a dead canker was noticed on a tree, no. 185 E, which had been injected with lithium hydroxide in April, May, and June, 1913, and in June and July, 1914. The dead canker was not noticed at first for the reason that dead bark covered the area (Pl. IV, fig. *A*). Not until this bark was removed (as one would remove the scab from a healed wound) was it noticed that a healthy callus had cut out the cankerous growth (fig. *B*). This same effect was noticed on a tree injected with sodium carbonate and on a thymol-injected tree. In 1916 these trees again became infected, and in 1917 the new chestnut blight cankers on them were growing at the normal rate.

In the meantime a better method of injecting the trees had been devised (Pl. IV, fig. *C*). Injections were made on forest trees as well as on small orchard trees.

In 1916 the injections were made with lithium and sodium salts only. The injections were made in three different regions. One set of trees was injected in April, May, and June, the second in June, July, and August, the third in August, September, and October. The results of these injections showed in 1917 that sodium salts were not as effective as lithium salts. The lithium injections made in April, May, and June seemed to have the greatest effect, in that the cankers were not growing vigorously and the trees had started to form a callus about the diseased areas. All the check trees were dead at the time of the inspection. Those injections made in August, September, and October appeared to have had the least effect. In no case had an injection definitely stopped the growth of a canker. No further inspection has been given these trees.

SUMMARY OF RESULTS

This and the preceding paper[6] constitute a report on an attempt made to answer by experimentation the following questions:

1. What substances can be injected into living chestnut trees?
2. When can they be injected?
3. Where does the injected material go?
4. What is the effect on the tree?
5. What is the effect on *Endothia parasitica* growing on the tree?

A compilation of the records of injections made in living chestnut trees during the growing seasons for five years showed:

1. That the trees possess a considerable capacity for absorbing solutions of substances. Solutions of organic compounds went into the trees more readily than solutions of inorganic compounds, the "true solutions" more readily than the colloidal. Injected solutions, with a very few exceptions, were absorbed more readily than injected water. In the dilutions used in these experiments, the more concentrated the solutions were, the more readily they were absorbed by the trees.

2. In southeastern Pennsylvania, June was the best month for injection in so far as rate of intake was concerned; then came July, May, August, September, October, and April. The rate of intake varied more in April, May, and June than in the summer and autumn months, but obviously was dependent upon the local weather conditions.

3. Examination of the trees showed that the injected solutions as a rule passed through the vessels of the youngest annual ring of wood up and down the tree trunk in a zone whose width was usually but little more than that of the injection hole. They passed into the branches and leaves, and in the case of the lithium salts into the nuts. They passed into the roots.

4. In general, the injection of the alkali metals was not detrimental to the trees; injection of heavy metals was detrimental; colloidal metals were not detrimental; organic compounds were not detrimental; water extract of chestnut blight canker was detrimental, healthy bark extract was not. The effect varied with the dilution of the solution and with the month in which the injection was made. Many of the bases produced characteristic discolorations of the leaves. Usually the visible effect of a solution upon a tree varied with the distance from the point of injection. The injections can cause the appearance of pathological xylem in the tree trunks.

5. This work is not completed and the results are inconclusive. Dilute

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[6] Rumbold, C. The injection of chemicals into chestnut trees. Amer. Journ. Bot. 7: 1–20. 1920.

solutions of lithium salts injected in the spring months may have an effect on the chestnut blight fungus in that the growth of the cankers on the injected trees appeared to be checked somewhat and the trees showed a tendency to form a callus about the canker.

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EXPLANATION OF PLATES III AND IV

Plate III

Fig. *A*. Tree no. 185E. An 8-year-old, grafted tree 4.85 m. high, 8 cm. in diameter. Injected April 15 to June 25, 1913, with 10 liters of lithium hydroxide 1/500 G.M. Leaf collected June 20, 1913. This tree produced many large nuts in the autumn. The shaded areas in the illustration indicate the brown portions of the leaf.

Fig. *B*. Tree no. 21C. A 16-year-old grafted tree 5.5 m. high, 1.1 cm. in diameter. Injected June 20 to October 16, 1913, with 26 liters of lithium carbonate 1/500 G.M. A diagrammatic drawing showing a cross section of portion of the trunk. *a*. Holes running up and down the trunk caused by the death of the cambium layer in the path of the injected alkali. *b*. The irregular year ring of wood formed during the injection period.

Fig. *C*. Tree no. 114E. A 9-year-old grafted tree, 46 m. high, 9 cm. in diameter. Injected May 9 to 15, 1913, with 14½ liters of meta-cresol 1/1000 G.M. The branch was cut August 15. Callus had formed along the edges of the paths of the solution. *a*. Diagrammatic drawing of cross-section of small branch. *b*. This year's ring of wood, normal in structure. *c*. The edge of the creosote stain. All tissue reached by the creosote was killed.

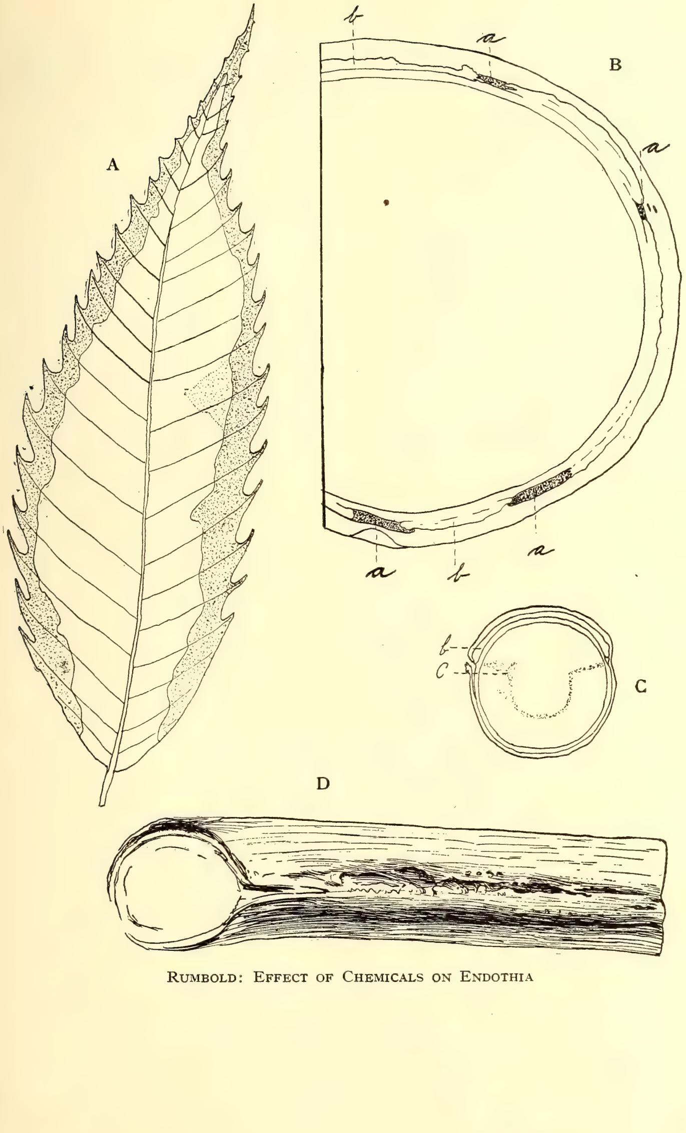
Fig. *D*. Tree no. 114E. Small branch showing an edge of a path of injected creosote solution. Normal callus separated the living bark tissue from the injected dead tissue.

Plate IV

Fig. *A*. Tree no. 185E. An 8-year-old grafted tree, 4.85 m. high, 8 cm. in diameter. Injected April 15 to June 25, 1913, with 10 liters of LiOH 1/500 G.M. Injected again June 11 to June 17, 1914, with LiOH 1/200 G.M., and from June 26 to July 27 with 2 liters of LiOH 1/100 G.M. solution. Tree inoculated with *Endothia parasitica* October, 1913. Canker photographed October, 1915, when it was noticed that the canker had stopped growing.

Fig. *B*. Tree no. 185E. Same canker as above, photographed in November, 1915, when the dead bark formerly covering the canker had been pulled off. The clean, healthy callus which had "cut out" the fungus was thus disclosed. On the side branch can be seen the check made at the time the tree was inoculated. At the base of the photograph can be seen the upper part of a canker caused by a natural infection at the fork of a branch. This canker also had been "cut out" by a callus.

Fig. *C*. A method of injecting trees of any diameter. Link chains tightened by turnbuckles hold the rubber corks to the trees. Glass T-tubes thrust through the corks introduce the liquid into the injection holes. A tempered steel tube shaped like a cork-borer makes the hole for the injected solution. It can be driven into the tree through the horizontal arm of the T-tube after the apparatus is in place. A piece of rubber tubing is put on the free end of the horizontal arm of the tube, and the solution is cut off with a pinchcock after the drill is removed.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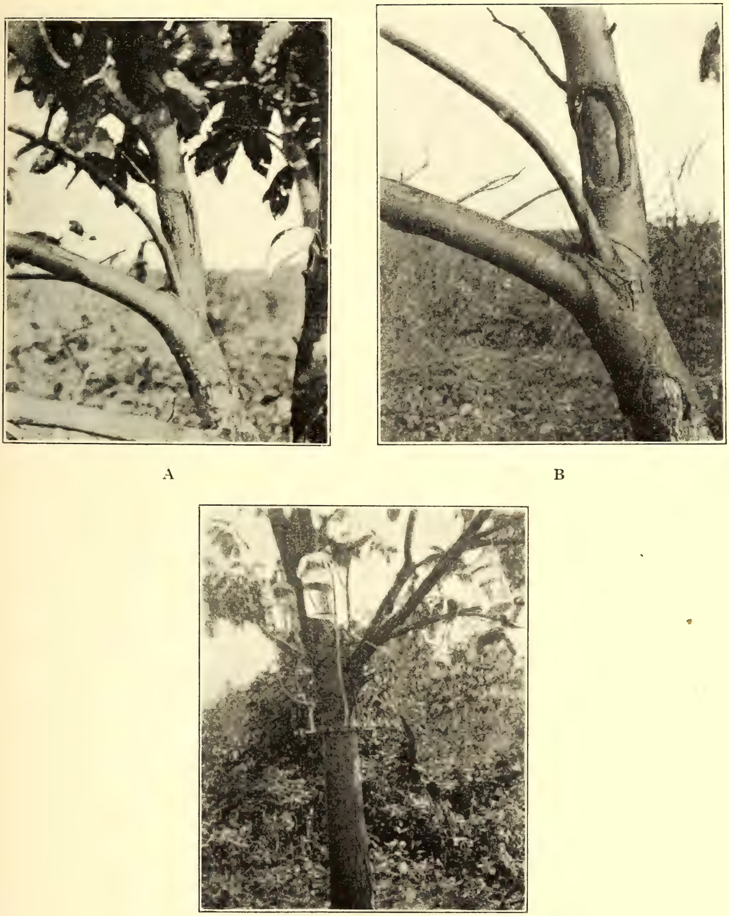
RUMBOLD: EFFECT OF CHEMICALS ON ENDOTHIA



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RUMBOLD: EFFECT OF CHEMICALS ON ENDOTHIA

SUBALPINE LAKE-SHORE VEGETATION IN NORTH-CENTRAL COLORADO

FRANCIS RAMALEY

INTRODUCTION

Very little has been published on the shore vegetation of lakes in Colorado. Brief references were made to lakes of the Pike's Peak region a number of years ago by Clements (1, 2). A somewhat extended account by the writer and W. W. Robbins (9) described the associations at Redrock Lake, Boulder County, Colorado, in the subalpine zone. Later, a short paper (5) pointed out certain features of shore vegetation in the montane zone. Recently Dr. Robbins has given a most careful and illuminating description (11) of a number of lakes in the montane zone near Tolland, Colorado. The present writer, dealing with sedges of northern Colorado, has named and characterized (7) certain of the plant associations of lake shores at different altitudes. In a paper by Dodds (3) on the plankton crustacea of Colorado lakes there are some references to vegetation and a very good account of physiography and climate.

The following pages give the results of a study of subalpine lakes, the study carried on chiefly from the University of Colorado Mountain Laboratory (8) at Tolland, Colorado, during the last ten years. A later paper will take up alpine lakes.

PHYSIOGRAPHY

The area in which lake-shore vegetation has been studied is a strip about 5 miles wide, along the eastern slope of the continental divide and extending from the southern boundary of the Rocky Mountain National Park south to Parry Peak, a distance of 24 miles. About 50 of the subalpine and alpine lakes of this district have been visited, and also a few on the western slope, in Grand County (see maps, figs. 1 and 2). The subalpine lakes more carefully studied are listed below together with altitudes in feet above sea level. These lakes are all of small size, the largest scarcely more than a half mile in length.

Lakes in Boulder County: Redrock Lake (10,100), Brainard Lake (10,350), Long Lake (10,500), Silver Lake (10,200), Emerald Lake (11,250), Dixie, or Jenny, Lake (11,000).

Lakes in Grand County: Corona Lake (11,165), Corona Reservoir (11,350), Lake Epworth (11,250).

Lakes in Gilpin County: Forest Lakes (10,800–10,900), Arapahoe Lakes (10,700–11,200), Crater Lakes (10,400–11,000), Echo Lake (11,072), James Peak Lake (11,090).

Lakes in Clear Creek County: Steuart Lake (11,350), Reynold's Lake (11,350), Loch Lomond (11,140).

The continental divide in the area studied is, for the most part, about 12,000 feet above sea level, with a few passes slightly lower and certain peaks much higher (Mt. Audubon, 13,225; Arapahoe Peak, 13,506; James Peak, 13,260; Parry Peak, 13,345). Long ridges extend out peninsula-like from the divide. Between the ridges are deep valleys all or most of which held glaciers at various points during comparatively recent times. On Arapahoe Peak there is still a permanent glacier of considerable size. A mile to the north is a smaller one (Henderson Glacier) and two others, also small, the Fair Glacier and the Isabelle Glacier, are four miles farther. At the extreme northern limit of our area of study are two more, the St. Vrain Glaciers. The broader parts of valleys are from one half to three quarters of a mile in width, and each may hold a group of small lakes, sometimes six or more. Along the flanks of ridges and on the slopes of the divide there are numerous cirques each with a single small lake.

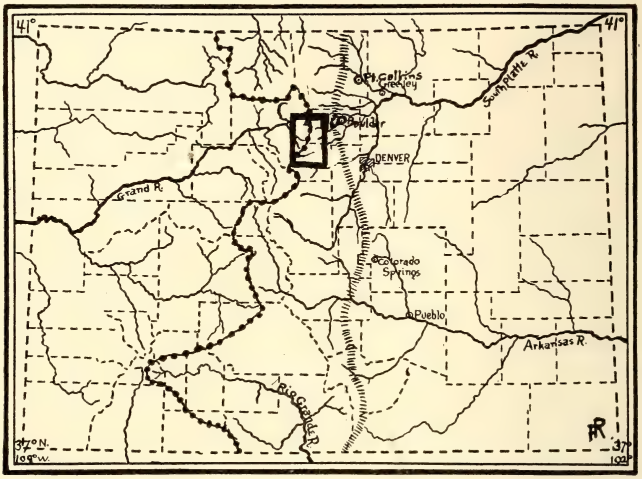

FIG. 1. Key map of Colorado. The Continental Divide is shown by the heavy dotted line, the front range of foothills by the short horizontal lines. The black rectangle outlines the area in which lake vegetation has been studied. Immediately north of this area is situated the Rocky Mountain National Park (not indicated on the map), of about the same size and shape as the part here marked out.

The higher lakes are chiefly of the rock-basin type while those of the subalpine zone are usually morainal. No ox-bow lakes are known to the writer above 10,000 feet altitude, although they occur in parks of the montane zone (8,500 to 10,000 feet).

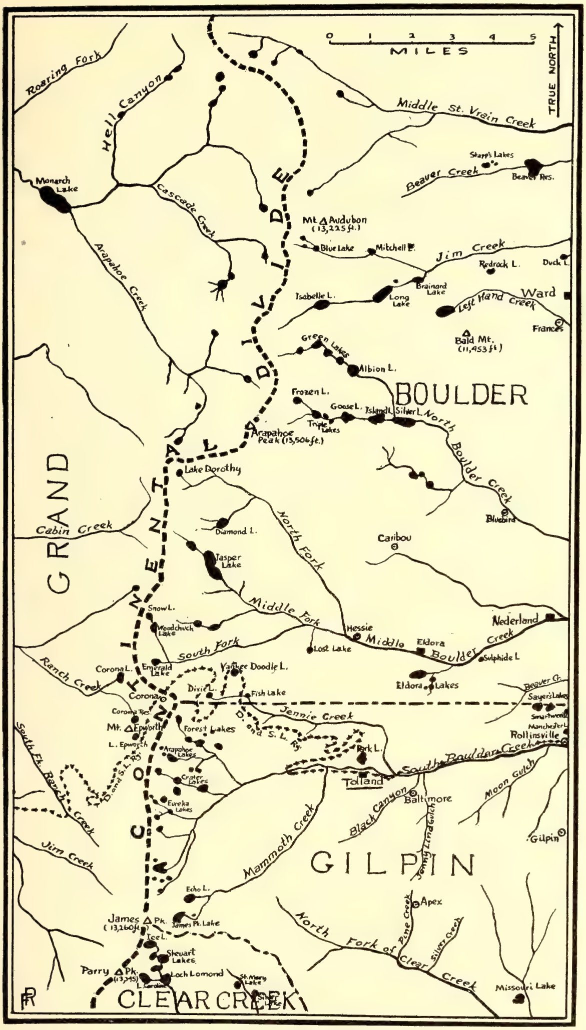
0 1 2 3 4 5
MILES
TRUE NORTH
Roaring Fork
Hell Canyon
Middle St. Vrain Creek
Stapp's Lakes
Beaver Creek
Beaver Res.
Monarch Lake
Cascade Creek
Mt. Audubon (13,225 ft.)
Blue Lake
Mitchell L.
Jim Creek
Redrock L.
Duck L.
Arapahoe Creek
Brainard Lake
Isabelle L.
Long Lake
Left Hand Creek
Ward
Frances
Bald Mt. (11,453 ft)
Green Lakes
Albion L.
BOULDER
Frozen L.
Goose L.
Island L.
Silver L.
North Boulder Creek
Triple Lakes
Arapahoe Peak (13,506 ft.)
CONTINENTAL DIVIDE
Lake Dorothy
GRAND
Cabin Creek
Diamond L.
North Fork
Bluebird
Caribou
Jasper Lake
Middle Fork
Snow L.
Woodchuck Lake
Hessie
Nederland
Middle Boulder Creek
Eldora
South Fork
Lost Lake
Sulphide L
Ranch Creek
Corona L.
Emerald Lake
Yankee Doodle L.
Eldora Lakes
Beaver Cr.
Dixie L.
Fish Lake
Sayer's Lakes
Corona
Jennie Creek
Smartweed
Corona Res.
D. and S. L. Ry.
Manchester L.
Mt. Epworth
Forest Lakes
Rollinsville
L. Epworth
Arapahoe Lakes
Park L.
South Fk. Ranch Creek
South Boulder Creek
Crater Lakes
Tolland
Black Canyon
Baltimore
Moon Gulch
Eureka Lakes
Mammoth Creek
Jenny Lind Gulch
Gilpin
Jim Creek
GILPIN
Echo L.
Apex
James Pk. (13,260 ft.)
James Pk. Lake
North Fork of Clear Creek
Pine Creek
Silver Creek
Ice L.
Steuart Lakes
Parry Pk. (13,345)
Loch Lomond
St. Marys Lake
L. Cardine
Missouri Lake
Silver Lake
FR
CLEAR CREEK

FIG. 2. Map of the area in which lake vegetation has been studied, *i.e.*, the part of the state included in the black rectangle of figure 1: portions of Grand, Boulder, Gilpin, and Clear Creek counties. The Continental Divide is in most places about 12,000 feet in altitude. Lakes close to the divide are of the alpine type; those from one to four miles away, and hence at lower altitudes, are generally of the subalpine type. The University of Colorado Mountain Laboratory located at Tolland, in Gilpin County, has served as the usual base from which trips were made on foot, but Ward, in Boulder County, was used as headquarters for the study of Redrock Lake.

CLIMATE AND SOIL

The climate of the area under consideration is cold, corresponding to that of Labrador, the Hudson Bay country, and southern Greenland. The timber limit is about 11,500 feet; lakes above this datum I shall speak of as alpine, those between this altitude and that of 10,000 feet as subalpine.

At Corona, on Rollins Pass, at an altitude of 11,660 feet, a government weather-bureau station was maintained (3) for a number of years. Here the mean annual temperature was found to be 26 degrees F. and the precipitation 43.7 inches. These figures may be taken as representative of the alpine region in this part of Colorado, although the tops of the higher peaks are, no doubt, colder and wetter. Throughout the alpine district there is frost every month of the year and in many places nearly every day.

For the subalpine zone only very incomplete records are available, but it is likely that at 10,000 feet the mean annual temperature is about 36 degrees F. and the precipitation 30 inches. The period without frost does not exceed three or four weeks even in favorable seasons. Precipitation is always ample during the growing period. There are frequent light showers during July and August (8). In table 1 a comparison is made with various points in the northern United States so that a clearer idea of the climate of our area of study may be gained.

TABLE 1. *Temperature and precipitation in the subalpine and alpine zones of Colorado compared with data from various points in the northern United States. Temperature in degrees Fahrenheit; precipitation in inches*

Station	Mean Annual Temperature	Mean July Temperature	Mean Annual Precipitation
Subalpine zone (10,500 ft.)	34.0[1]	54.0[1]	32.0[1]
Alpine zone (Corona, 11,660 ft.)	26.0	47.0	44.0
Denver, Colo. (5,275 ft.)	49.8	71.8	14.0
St. Paul, Minn.	45.0	74.0	28.6
Duluth, Minn.	39.0	66.0	29.9
Chicago, Ill.	48.0	72.0	33.4
New York, N. Y.	52.0	74.0	44.8

The temperature of the soil, as would be expected, is low. Numerous observations have been made at subalpine lakes. Readings at 3 dm. depth are shown in table 2. In analogous associations at ordinary altitudes in the northern United States the temperatures are 10 to 18 degrees higher.

TABLE 2. *Soil temperatures of subalpine lake shores for July; average of numerous observations at 3 dm. depth, in degrees F.*

Subalpine sedge moor, near water	50
Subalpine meadow, on higher ground	52
Subalpine spruce forest (dense)	48
Subalpine forest openings (dry places)	60

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[1] Data very meager; estimated by comparison with stations in the alpine and montane zones.

Soils throughout the area studied are derived primarily from granitic rocks. On many ridges the material is a compact disintegrated granite, and the shores of subalpine lakes are often of this material interspersed with large and small boulders. At inlets and outlets, and wherever an accumulation of wash from adjacent slopes occurs, the soil is a black loam. Here sedge moor and willow moor (willow scrub) develop. Typical subalpine meadow is found on lighter, better-drained soil, a sandy loam which occurs often as a circum-area of lakes between the moor and the forest.

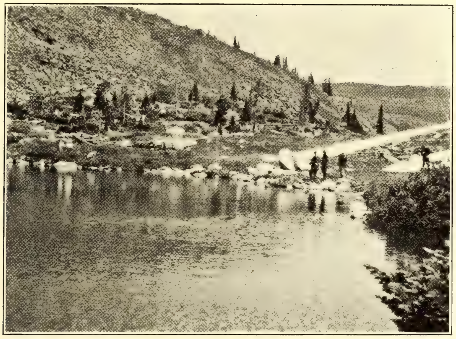

FIG. 3. Part of Corona Lake (altitude 11,165 feet), a high subalpine lake without any complete circum-areas of vegetation. Numerous large rocks are to be noted along the shore which show that there has been very little infilling. In the lower right-hand corner of the picture an Engelmann spruce is seen; behind this is a clump of willows; farther around is sedge moor; then more willows.

Soil moistures have not been so fully determined as soil temperatures. Figures for July, 1918, at Redrock Lake (altitude 10,100 feet) are, however, available (9). They indicate an abundance of moisture. Averages are shown in table 3.

TABLE 3. *Soil moisture percent at 3 dm. depth during July, 1908, at Redrock Lake in the subalpine zone*

Subalpine sedge moor, near water	65
Subalpine meadow, on higher ground	21
Subalpine spruce forest (dense)	29
Subalpine forest openings (dry places)	7

No studies of wilting coefficients have been made, but so far as the writer's observations go there is little wilting of vegetation even in the driest weather.

Probably the chief limiting factors for plant growth around subalpine lakes are low temperature, extreme shortness of season, and shallow soil.

At very high altitudes there can be no doubt that the drying effect of winter winds and the heavy snows are important in preventing forest development.

PLANT COMMUNITIES OF LAKE SHORES

Types of Zonation

Great similarity exists in the shore vegetation of the various lakes studied. It is convenient, as already suggested, to separate subalpine and alpine life-zones by an arbitrary datum of 11,500 feet altitude. There is, however, no sharp difference in vegetation immediately below and above this line, yet it is possible to distinguish a subalpine and an alpine type of lake. The former only will be considered in the present paper. So far as the writer is able to do so, he will use a terminology consonant with that proposed by Nichols (4). Plant nomenclature will be that of Rydberg (12).

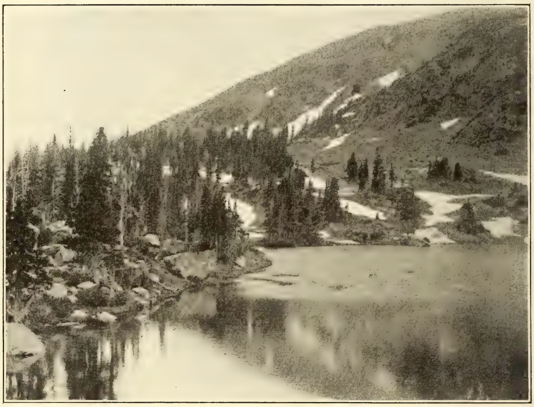

FIG. 4. One of the larger Forest Lakes (altitude 10,800 feet), showing coniferous orest of Engelmann spruce coming down close to the lake edge. There has been very little infilling either through wash from the slopes or through accumulation of plant remains. At the left there is a narrow fringe of moss moor; on the shore opposite the observer a considerable amount of meadow moor has developed in the lower places close to shore. Photograph in early June by Dr. W. W. Robbins.

Subalpine lakes are typically surrounded by Engelmann spruce forest in which subalpine fir (*Abies lasiocarpa*) and lodgepole pine (*Pinus murrayana*) may occur in small amount. Aspens (*Populus tremuloides*) are occasionally present, but they belong rather to the montane zone where they are very abundant. On the eastern wind-swept shores of lakes the forest is often made up largely of limber pines (*Apinus flexilis*), the trees scattered with intervening open spaces.

Lakes shut in by steep slopes often show no special shore vegetation. The Engelmann spruce forest extends down close to the lake edge, and here there may be a zone of rocks with almost no vegetation at all. This condition obtains particularly where the lake level fluctuates from year to year or in different months of the same season. Most lakes have some parts of the shore either barren or else covered with forest. Lakes which show no true shore vegetation furnish nothing for discussion in the present paper.

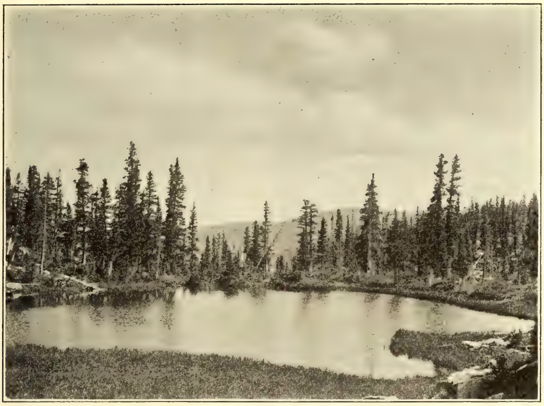

FIG. 5. One of the small Forest Lakes (altitude 10,800 feet); a shallow pond with much infilling and showing complete circum-areas of moss moor and sedge moor. A half submersed belt of *Carex aquatilis* runs about one third of the way around the lake. Nearly every one of the various lake-shore associations is represented in some part of the area surrounding this lake. Photograph by Dr. W. W. Robbins.

Many lakes have gently sloping banks for a part of their circumference. On these more moderate slopes a true shore vegetation develops, determined by differences from the forest in soil quality, soil moisture, and soil temperature. A common arrangement of the vegetation of these lakes is in three clearly-marked circum-areas (8). As a rule, some of the associations are absent at certain points. Willows may be present only near the lake inlet and outlet (see table 4).

TABLE 4. *Subalpine shore vegetation; condensed classification*

1. Moor; next to water:
    - *a.* Sedge moor; chiefly Carex, with Caltha, Bistorta, Clementsia, and other marsh plants.
    - *b.* Willow moor (willow scrub); farther from the water but the soil very wet. The herbaceous vegetation is chiefly Carex with some shade-enduring grasses and dicotyledons.
2. Meadow; on drier ground, but the soil fairly deep and of moderately fine texture. Often this is a close association of many species of flowering herbs.
3. Forest; Engelmann spruces chiefly, if the soil is deep, but lodgepole pine and limber pine on steeper slopes and on ridges with scanty soil.

In favorable parts of subalpine lake shores a closer analysis of the vegetation can be made than is indicated in table 4. Thus the moor is seen to include as many as six communities arranged in successive belts; a heath association may be distinguished outside the moor, *i.e.*, in drier ground; two or more consociations may form distinct bands in the meadow association. All of these communities are associated with differences in edaphic conditions and are not merely floristic in nature. Such a vegetation complex is indicated in table 5.

TABLE 5. *Subalpine shore vegetation, extended classification*

1. Moor (moor-association type):
    - *a.* Half submersed association of *Carex aquatilis.*
    - *b.* Moss-moor consociation; sedge moor with large amount of moss.
    - *c.* Typical sedge-moor association.
    - *d.* Willow-moor association; sedge moor with shrubby willows.
    - *e.* Rush-moor society; sedge moor with rushes (*Juncus Drummondii* and *Juncus mertensianus*).
    - *f.* Meadow-moor consociation; sedge moor with a number of meadow plants and hence a smaller proportion of Carex than typical sedge moor.
2. Heath association:
    - *a.* Heath moor, a transition between heath and moor.
    - *b.* Kalmia heath consociation.
    - *c.* Gaultheria heath consociation.
3. Meadow association:
    - *a.* Erigeron-Castilleja-Ligusticum consociation.
    - *b.* Pedicularis-Vaccinium consociation.
4. Forest association.

It seldom occurs that all these communities can be distinguished for any great distance along the shore. Many lakes have a part of the shore in which the vegetation analysis may be carried as far as indicated in our table, other parts of the shore may show no more than is suggested in table 4, while still other parts have no distinct shore vegetation at all. An abnormal position of some of the communities is often brought about, due to local areas of seepage. Islands of meadow moor occur in many places surrounded by sedge moor, while similar islands of willow moor and moss moor are common (see map, fig. 6). Meadow moor, rush moor, and the entire heath association are absent from many lakes. The positions of heath and meadow are sometimes completely reversed, or there may be heath meadow in which there is a mingling of plants of the two associations. Lakes near to timber limit may have little vegetation except meadow moor, or sedge moor and meadow moor.

Descriptions of the Various Lake-shore Zones (Circum-areas)

The *half submersed Carex aquatilis association* is typically a pure stand of the species of Carex which gives its name. This is in contrast to conditions in montane lakes (5, 7, 11), where three or more species may make up the half submersed zone.

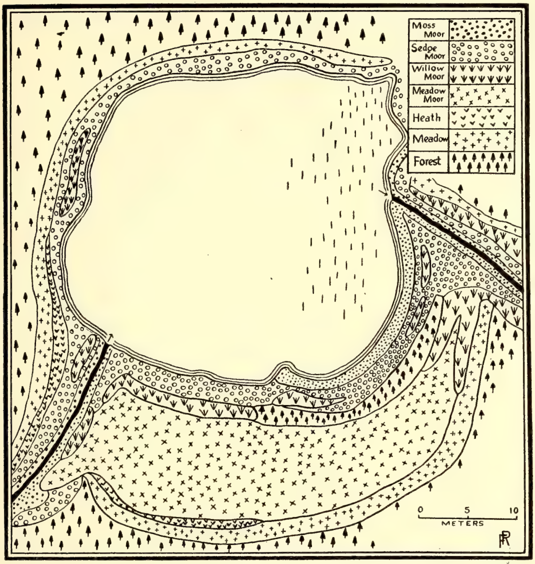

FIG. 6. Map of vegetation at Burgrass Lake, one of the Forest Lakes, a small subalpine pond at an altitude of 10,800 feet. Scattered plants of burgrass (*Sparganium angustifolium*) at the east side near the outlet give the lake its name. Rush moor and heath moor, imperfectly developed in places, could not well be shown on the map.

Typical *sedge moor* has often been described (5, 7, 9). *Moss moor* and *true sedge moor* may be distinguished in the following quadrat records in which the percentage of ground covered by each kind of plant is indicated (tables 6 and 7). Naturally, any quadrat in a community would be different from every other quadrat as to minute details, but quadrat records afford the best means of distinguishing similar communities.

TABLE 6. *Meter quadrat in subalpine moss moor at Burgrass Lake (one of the Forest Lakes) in Gilpin County, Colorado, August 17, 1918. The figures indicate percentages of ground covered*

Bare ground	0
Mosses	40
Carex aquatilis	40
Clementsia rhodantha	5
Ligusticum tenuifolium	5
Caltha rotundifolia	5
Salix chlorophylla	2
Viola palustris	1
Epilobium anagallidifolium	1
Dodecatheon radicatum	1
	100

TABLE 7. *Meter quadrat in true subalpine sedge moor at Burgrass Lake (one of the Forest Lakes) in Gilpin County, Colorado, August 17, 1918. The figures indicate percentages of ground covered*

Bare ground	0
Carex aquatilis	60
Mosses	15
Carex nigricans and *C. illota*	5
Caltha rotundifolia	5
Elephantella groenlandica	5
Ligusticum tenuifolium	5
Salix spp.	4
Viola palustris	1
	100

Willow moor, or willow scrub, as previously suggested, is sedge moor with willows in it. The writer has usually thought of it as quite distinct from sedge moor, but on subalpine lake shores no sharp distinction can be drawn. It does not form, as a rule, a clearly marked circum-area, as is common in the montane region below 10,000 feet. Where present at subalpine lakes, willow moor is often a stage preceding the development of Engelmann spruce forest. This is especially true in late stages of infilling, when a whole lake may become willow moor and, later, spruce forest. Willows are most likely to develop on deep fine-grained soil. The species are *Salix chlorophylla*, *S. glaucops*, *S. lutea*, and *S. Barclayi.*

Rush moor is a society of sedge moor. On many shores it is very conspicuous as a narrow belt outside the ordinary sedge moor, most often when willows are absent. The usual species of rush are *Juncus Drummondii* and *J. mertensianus*, with, sometimes, *Juncoides spicatum.*

Meadow moor is sedge moor with some meadow plants. It may form a definite belt between moor and meadow, but more often it occurs as large or small patches developed on soil somewhat elevated above ordinary sedge moor and hence better drained. According to the writer's "soil moisture index" (6), ordinary xerophytes are given the number 4, mesophytes 6, marsh plants 8, and aquatics 10. Meadow moor would be assigned 7 as its index number. It is sometimes convenient to call it a "no. 7 meadow." There is less sedge and especially less moss than in ordinary sedge moor. More different species of plants occur than in ordinary sedge moor or in moss moor. The following are likely to be present in considerable amount: *Ligusticum tenuifolium*, *Erigeron salsuginosus*, *Bistorta bistortoides*, *Arnica fulgens* and *A. subplumosa*, *Deschampsia atropurpurea*, *Senecio blitoides.* Besides these, almost any of the true meadow plants may occur.

The *heath association* is here a mere suggestion of the heaths so prominent in the shore vegetation of many lakes in the northeastern United States and in Canada. The writer designates as "heath moor" the transition between moor and heath. This may be a distinct belt in which plants of the two associations are mingled, or it may be entirely lacking. In the latter case the belt of heath may follow abruptly after sedge moor or rush moor or meadow moor. It may be, in turn, followed on higher ground by forest, or there may be an intervening strip of meadow. Heath may occur in scattered patches on elevated areas of the sedge moor, probably most often in shallow soil over large rocks. A quadrat record (table 8) taken in a part of the heath which is not differentiated into consociations gives an idea of the floristic composition.

TABLE 8. *Meter quadrat in subalpine heath association at North Forest Lake (altitude 10,800 ft.) in Gilpin County, Colorado, August 17, 1918. The figures indicate percentages of ground covered*

Bare ground and rocks	20
Gaultheria humifusa	25
Vaccinium caespitosum	18
Kalmia microphylla	15
Erigeron salsuginosus	7
Carex festivella et spp.	5
Ligusticum tenuifolium	4
Juncus Drummondii	2
Agrostis humilis	2
Lichens	2
	100

The *Kalmia heath consociation* is characterized by the low shrub *Kalmia microphylla*, about 2 dm. tall. This is often only scantily distributed, but sometimes it forms a clearly-marked though narrow belt of vegetation part way around the moor. The writer has, in no case, seen a complete circum-area of this plant.

The *Gaultheria heath consociation* seems to develop often on shallow soil. In it dense patches of *Gaultheria humifusa* occur. The plant is a depressed undershrub only a few centimeters high. Subordinate species are *Vaccinium caespitosum, Muhlenbergia filiformis, Erigeron salsuginosus, Hieracium gracile.* Almost any meadow plant may occasionally be present.

The *meadow association* is conspicuous because of the large and brightly-colored flowers of some of the abundant species. It would be possible to name six or more societies of local or infrequent occurrence distinguished by floristic differences, but in the present paper it will be best merely to characterize two consociations. These, as already indicated in table 5, may be called the Erigeron-Castilleja-Ligusticum consociation and the Pedicularis-Vaccinium consociation. They are rather constant in occurrence, the first named being next to the heath (or to sedge moor or rush moor or meadow moor in some cases), the second merging into the undergrowth of the forest. There may be considerable bare ground (10 to 40 percent). Many species characteristic of lake-shore meadows in the montane zone (10, 11) are absent, as *Fragaria glauca, Tium alpinum, Potentilla pulcherrima, Erigeron macranthus.*

The *Erigeron-Castilleja-Ligusticum consociation* of the meadow develops on fine-grained soil with abundant moisture, and well drained. The dominant species are *Erigeron salsuginosus*, *Castilleja confusa* and *C. lauta*, and *Ligusticum tenuifolium*. Among other plants are *Potentilla diversifolia*, *Amarella strictiflora*, *Achillea lanulosa*, *Artemisia saxicola* and *A. scopulorum*, *Veronica Wormskjoldii*, *Phleum alpinum*, *Carex festivella*, *Bistorta bistortoides*, *Antennaria umbrinella*, and those mentioned in the following paragraph.

The *Pedicularis-Vaccinium consociation* is on drier ground than the community just described. *Pedicularis Parryi* and *P. Grayi* are important. *Vaccinium oreophilum*, a typical plant under Engelmann spruces, is usually present here at the edge of the forest. *Vaccinium scoparium* and *V. oreophilum* may also be present. All of these species of Vaccinium may at times be found sparingly in the Kalmia heath consociation. Many subordinate species of the Pedicularis-Vaccinium consociation are plants common in the forest or in forest openings, such as *Thlaspi Nuttallii*, *Chamaenerion spicatum*, *Koeleria gracilis*, *Micranthes rhomboidea*, *Aquilegia coerulea*. Then there are such meadow plants of the montane zone as *Dasystephana Parryi* and *Troximon glaucum*, and such plants of alpine meadow as *Trifolium dasyphyllum* and *T. nanum*. In addition there may be any of the species mentioned in the preceding paragraph.

TABLE 9. *Meter quadrat in subalpine meadow at Burgrass Lake (one of the Forest Lakes) in Gilpin County, Colorado, August 17, 1918. The figures indicate percentages of ground covered*

Bare ground and rocks	10
Ligusticum tenuifolium	12
Erigeron salsuginosus	8
Castilleja confusa	7
Castilleja lauta	7
Artemisia scopulorum and *A. saxicola*	6
Potentilla diversifolia	5
Arnica fulgens	5
Agrostis humilis	5
Mosses	5
Amarella plebeja	4
Veronica Wormskjoldii	4
Vaccinium caespitosum	4
Hieracium gracile	3
Viola bellidifolia	3
Phleum alpinum	3
Sibbaldia procumbens	3
Trifolium dasyphyllum	3
Chamaenerion spicatum	2
Juncus Drummondii	1
	100

An idea of the composition of the meadow may be gained from a quadrat record at Burgrass Lake. Where this was taken the belt of meadow was so narrow that the meter quadrat covered both consociations, and most of the common meadow plants are, therefore, represented. Ligusticum, it will be seen, occupied more space than any other species. Castilleja and Erigeron were, however, more conspicuous because of their brilliant flowers.

The *forest association* surrounding subalpine lakes does not differ from the forest elsewhere in the same locality. It is, therefore, not included in the present study.

LIST OF PLANT SPECIES

The following list includes only the more frequent plants of subalpine lake shores. Certain species may be locally abundant and yet not widely distributed. These have generally been excluded. Plants belonging primarily to the forest and only occasionally getting in among the true moor, heath, and meadow plants are also not admitted, nor have aquatics been listed. A number of species characteristic of stream banks and of narrow gulches, as *Cardamine cordifolia*, *Heracleum lanatum*, *Primula Parryi*, *Mertensia ciliata*, and *Senecio triangularis*, are occasional in the moor but are not included in the list. Mosses, lichens, and fungi are not considered.

Many plants are lacking which are characteristic of lake shores at lower altitudes in Colorado and at ordinary altitudes in the United States east of the Rocky Mountains. The following may be mentioned: Equisetum, Typha, Alisma, Beckmannia, Panicularia, Cyperus, Scirpus, Iris, Populus, Betula, Persicaria, Rumex, Thalictrum, Rosa, Lathyrus, Vicia, Euphorbia, Menyanthes, Mentha, Prunella, Galium, Sambucus, Aster, Bidens, Iva, Rudbeckia, Solidago, Taraxacum. Some few of the above named are found in the shore vegetation of subalpine lakes close down to the 10,000-foot-altitude line, as Equisetum, Betula, Rosa. They do not, however, belong to typical subalpine lakes.

Since the soil moisture requirement is the most useful single feature to be known about a plant, provided the general climatic features of the region are known, this has been indicated in the list. The plan followed is that employed by the writer (6) and by one of his students (10) whereby, as previously stated, the figure 4 is used as the "soil moisture index" for ordinary xerophytes, 6 for mesophytes, 8 for marsh plants, 10 for aquatics. Most plants of sedge moor have a soil moisture index of 8, most plants of meadow have a soil moisture index of 6. Species which grow under various conditions are given more than one number.

POACEAE

Agrostis humilis (6, 7)
Alopecurus occidentalis (7, 8)
Deschampsia alpicola (6, 7, 8)
Deschampsia caespitosa (7, 8)
Koeleria gracilis (5, 6)
Muhlenbergia filiformis (6, 7)
Phippsia algida (7)
Phleum alpinum (7)
Poa alpina (7, 8)
Poa subpurpurea (5)
Sporobolus brevifolius (6)

CYPERACEAE

Carex albo-nigra (7, 8)
Carex aquatilis (8, 9)
Carex ebenea (7)
Carex festivella (6)
Carex nigricans (6, 7, 8)
Carex pyrenaica (6)
Carex rostrata (8, 9)
Carex scopulorum (8)

JUNCACEAE

Juncoides spicatum (5, 6, 7)
Juncus Drummondii (6, 7)
Juncus mertensianus (7, 8)

SALICACEAE

Salix Barclayi (8)
Salix chlorophylla (8, 9)
Salix glaucops (7, 8)
Salix lutea (8)

POLYGONACEAE

Bistorta bistortoides (7, 8)
Bistorta vivipara (7, 8)

ALSINACEAE

Alsine longifolia (7)

RANUNCULACEAE

Aquilegia coerulea (6)
Caltha rotundifolia (7, 8, 9)
Ranunculus alismaefolius (8)
Ranunculus alpeophilus (7, 8)
Trollius albiflorus

BRASSICACEAE

Draba Parryi (5, 6)
Thlaspi Nuttallii (5)

CRASSULACEAE

Clementsia rhodantha (8, 9)

SAXIFRAGACEAE

Micranthes arguta (8, 9)
Micranthes rhomboidea (6)

ROSACEAE

Dasiphora fruticosa (5, 6, 7)
Potentilla diversifolia (7)
Sibbaldia procumbens (6)

FABACEAE

Trifolium dasyphyllum (5, 6)
Trifolium nanum (5, 6)
Trifolium Parryi (5, 6)

VIOLACEAE

Viola bellidifolia (6)
Viola palustris (8)

ONAGRACEAE

Chamaenerion spicatum (5, 6)
Epilobium alpinum (8, 9)
Epilobium anagallidifolium (8, 9)
Epilobium Hornmannii (8)

AMMIACEAE

Angelica Grayi (8)
Ligusticum tenuifolium (7, 8)
Oxypolis Fendleri (7, 8)

ERICACEAE

Gaultheria humifusa (6, 7)
Kalmia microphylla (7)

VACCINIACEAE

Vaccinium caespitosum (5, 6)
Vaccinium oreophilum (5, 6)
Vaccinium scoparium (5, 6)

PRIMULACEAE

Androsace subumbellata (6)
Dodecatheon radicatum (8, 9)

GENTIANACEAE

Amarella monantha (8)
Amarella plebeja (7)
Amarella strictiflora (7, 8)
Chondrophylla Fremontii (8)
Dasystephana Parryi (6)
Dasystephana Romanzovii (6, 7)
Pleurogyne fontana (8)
Swertia scopulina (8, 9)

SCROPHULARIACEAE

Castilleja confusa (6)
Castilleja lauta (7)
Elephantella groenlandica (8)
Pedicularis Grayi (6)
Pedicularis Parryi (6, 7)
Pedicularis racemosa (5, 6)
Pentstemon stenosepalus (6)
Veronica Wormskjoldii (8)

CAMPANULACEAE

Campanula petiolata (5, 6, 7)

CARDUACEAE

Achillea lanulosa (5, 6, 7)
Antennaria umbrinella (6)
Arnica fulgens (6)
Arnica Parryi (6)
Arnica subplumosa (6)
Artemisia saxicola (6)
Artemisia scopulorum (6)
Erigeron salsuginosus (6, 7)
Oreochrysum Parryi (5, 6)
Senecio blitoides (6)
Senecio crassulus (6, 7)

CICHORIACEAE

Agoseris glauca (5, 6)
Hieracium gracile (6)

SEASONAL ASPECTS

The growing period of subalpine lake-shore vegetation may be separated (9) into three seasons: vernal (May 15 to July 1), estival (July 1 to August 15), autumnal (August 15 to October 1).

During the vernal period there are few lake-shore plants in bloom. About June 1 or a little later the willow catkins appear. The brown of winter continues, especially at higher altitudes, until the middle of June. There are still large snowdrifts in the forest and often some in the open. It must not be thought that there is no fresh vegetation in the subalpine zone at this time. A number of xerophytic plants of ridges and dry slopes and forest openings are in bloom, but these do not belong to our present study. Of lake-shore plants, in addition to the willows already mentioned, the spring bloomers are Caltha, Trollius, *Viola bellidifolia*, *V. palustris*, *Thlaspi Nuttallii*, *Draba Parryi*. Caltha and Trollius, on account of their abundance and their showy flowers, are the conspicuous plants of the spring season.

During the estival period the great majority of lake-shore plants come into blossom. Many of the sedges are rather early; grasses are somewhat later. The spring flowers continue for a time but are soon overshadowed by the abundance and brilliancy of the summer bloomers. In the moor there are Clementsia, Dodecatheon, Elephantella, Dasiphora, Swertia, and Senecio. Among meadow and heath plants may be mentioned the Castillejas, the Arnicas, *Potentilla diversifolia*, Kalmia, Campanula, *Erigeron salsuginosus*, *Hieracium gracile*.

During the autumnal period the summer-blooming plants are in fruit and a few of them continue to bloom for a time, especially *Erigeron salsuginosus*, *Campanula petiolata*, the Castillejas, and the Arnicas. True autumn-blooming plants are the gentians Amarella, Chondrophylla, and Dasystephana, also *Antennaria umbrinella* and *Ligusticum tenuifolium.* The snows which usually come about the first week in September melt away in a few days, and some of the plants continue in bloom up to about September 20, or occasionally even to October 1.

SUCCESSIONS

The character of succession on the lake shores here described is so obvious that an extended discussion will not be necessary. It needs to be kept in mind that the topography of the area is "new." There is comparatively little soil anywhere except in depressions where it has been washed down from adjacent slopes. Lake bottoms are typically of boulders. Climate is of the cold, moderately wet type which favors the development of coniferous forest. Engelmann spruce forest is the ultimate climatic association and appears wherever edaphic conditions are at all favorable.

A few subalpine lakes, especially those at lower altitudes close to 10,000 feet, have a moderate amount of aquatic vegetation, chiefly *Potamogeton* spp. and *Sparganium angustifolium.* These plants contribute slightly to the filling up of lakes. At higher altitudes they are generally absent and the only lake vegetation of consequence is Carex, which may form in places a strip of half submersed plants extending into the water from the moor. The Carex here is commonly *C. aquatilis*, which is also the chief sedge of the moor that surrounds subalpine lakes.

Often the vegetation advances very slowly into the lake because of the heaving action of ice. This thrusts itself into the bank and destroys vegetation. It may give rise to an elevated "rim" as shown by Robbins (11), projecting out a few decimeters over the edge of the water.

Infilling of subalpine lakes is, it will be recognized, largely a physiographic process depending upon stream sediment chiefly near the inlet, or upon storm wash from surrounding slopes. When, however, a lake has once been filled so that the water is quite shallow, then a rapid invasion by Carex may occur.

The normal positions of the various plant communities show their successional relations. Half submersed Carex association is followed by moor, and this by heath and meadow and forest. When level ground is raised by accumulation of plant remains or by alluvial wash then a later successional stage of vegetation develops. The same change occurs when the lake level is lowered. One or more stages may be skipped.

Moss moor is usually the wettest part of the moor. As vegetation advances this becomes drier and changes to ordinary sedge moor or to meadow moor. The willow moor stage may follow sedge moor if there is a good depth of fine-grained soil. While in montane situations willows tend to form a complete circum-area of lakes, they are relatively unimportant in the shore vegetation of the subalpine region. Where they do occur they may initiate a shortened successional series, for they permit the establishment of Engelmann spruce without the intermediate heath and meadow stages.

"Dry forest" of limber pine and lodgepole pine does not follow meadow but belongs to the xerarch series, developing on ridges and on rough, stony ground. In time, except where winds are so severe as to blow away the humus, Engelmann spruce forest, as the ultimate climatic association, will replace the dry forest.

SUMMARY

The subalpine zone, 10,000 to 11,500 feet in altitude, in north-central Colorado has a large number of small lakes, some of morainal, some of rock-basin type. These have a characteristic shore vegetation often developing definite circum-areas. The climatic climax association is Engelmann spruce forest, and the stages of succession in the filling up of a lake lead eventually to this forest. Very few aquatic plants occur, but there is sometimes a circum-area (usually incomplete) of half submersed sedges. Following this there may be in the more complete cases: (1) a well developed moor composed largely of Carex, sometimes separable into moss moor, sedge moor, willow moor, rush moor, and meadow moor; (2) a heath association of *Kalmia microphylla* and *Gaultheria humifusa;* (3) meadow association in which the principal plants are species of Erigeron, Castilleja, Ligusticum, Pedicularis, and Vaccinium; (4) forest association, dominated by Engelmann spruce. The various associations and their numerous subdivisions are described by the author together with their successional relations and seasonal aspects. A sketch is given of topography, climate, and soil, and a list is made of the characteristic lake-shore plants, with soil-moisture index of each. The paper is based upon the study of a large number of subalpine lakes in four different counties of north-central Colorado.

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SOME OBSERVATIONS ON THE SPORE DISCHARGE OF PLEURAGE CURVICOLLA (WINT.) KUNTZE.

J. L. Weimer

Introduction

While making some studies on the comparison of a strain of *Pleurage curvicolla* (Wint.) Kuntze with those strains previously investigated, the results of which studies are being published in a separate paper,[1] it was noticed that this organism has the power to discharge its spores with remarkable force.

So far as known, parasitic Ascomycetes are able to project their spores only to a distance of a few millimeters, while it has been shown that certain saprophytic forms such as members of the Sordariaceae, Ascobolaceae, and others are able to shoot their spores a considerably greater distance. Griffiths[2] studied the method of spore discharge in *P. curvicolla* as well as in related species, and found that the maximum height of projection in the former is 9 centimeters. A good discussion of the details of spore ejection by these fungi is given by Griffiths and need not be repeated here. Woronin[3] found that *Sordaria fimiseda* could shoot its spores to a height of 15 centimeters. This is the maximum distance found recorded for a member of the Sordariaceae.

Buller[4] states that *Ascobolus immersus* is possibly not exceeded by any other Ascomycete in the violence of its spore discharge. It was found to project its spores to a height of 35 centimeters.

Experiments

In the experiments recorded below, the fungus was grown on moistened corn meal in a two-liter Erlenmeyer flask. The cultures were allowed to grow at room temperature, about 24–28° C., and exposed to the diffused light of the laboratory until the perithecia began to discharge their spores as indicated by the presence of the spore masses upon the sides of the flask. It might be mentioned in passing that few and often no perithecia were found in cultures grown in an incubator in the dark or wrapped in black paper, while in duplicate cultures grown in the laboratory in the light, perithecia were formed in abundance. When the culture was ready for use the cotton stopper was removed and a large test tube, 37 mm. in inside diameter and 243 mm. in height, was inverted over the mouth of the flask. The flask and all but the bottom of the tube were covered with black paper, to keep out the light. The apparatus was placed upon a desk before a north window, where it was exposed to rather strong diffused light. The perithecia are positively heliotropic, and hence the spores were discharged towards the source of light, namely, the bottom of the test tube. Spore masses were found all along the sides of the test tube to a height of 45 cm. above the fruiting surface of the culture. None were found on the bottom of the tube 5 cm. higher. This is three times as high as previously recorded for a member of this family, and 10 cm. higher than given by Buller for *Ascobolus immersus*. So far as the writer has knowledge, this strain of *Pleurage curvicolla* can project its spores to a greater height than any other Ascomycete yet studied and three times as high as any other Pyrenomycete investigated.

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[1] Weimer, J. L. Variations in *Pleurage curvicolla* (Wint.) Kuntze. Amer. Journ. Bot. **6**: 406–409. 1919.

[2] Griffiths, David. The North American Sordariaceae. Mem. Torrey Club **11**: 1–134. 1901.

[3] Woronin, M. *Sphaeria Lemaneae, Sordaria fimiseda, Sordaria coprophila* und *Arthrobotrys oligospora.* Abhand. Senkenb. Naturforsch. Gesell. (Frankfurt) **7**: 325–360. pl. 1–6. 1869–1870.

[4] Buller, A. H. R. Researches on fungi. pp. 287. 1909.

Considerable attention has been paid by various workers to the study of the influence of light upon the direction in which spores are discharged. Allen and Jolivette[5] have reported in detail, observations made on the accuracy with which Pilobolus could project its sporanges toward a source of light. While endeavoring to determine the height to which *Pleurage curvicolla* could shoot its spores, the writer incidentally made a few observations on its power to aim towards the light. Spore masses have often been seen on the side of the petri dish in which this organism was growing next to the source of light. In most cases perithecia were present only at or near the center of the culture, and these had oriented their beaks in such a way as to cause the spores to be discharged in a direction parallel to the surface of the substratum on which they were growing.

In one experiment a two-liter flask containing the fungus growing on corn meal was wrapped in two thicknesses of black paper, and a hole about 2½ cm. in diameter was cut in the paper on the side of the flask nearest the source of the strongest light. After 48 hours a considerable deposit of spore masses was present on the side of the flask immediately beneath the opening in the paper. The flask was then turned about so that the hole in the paper was directly opposite where it had been. This would mean, of course, that many of the beaks of the perithecia were pointing directly away from the opening. After a period of 48 hours a considerable deposit of spores was present beneath the opening in the paper. The paper was moved in this manner time after time with the result that each time the perithecia changed their aim and discharged their spores towards the source of light.

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[5] Allen, Ruth F., and Jolivette, Hally D. M. A study of the light relations of Pilobolus. Trans. Wisc. Acad. Sci., Arts, and Lett. **17**: 533–598. 1914.

In another experiment the opening in the paper was 1½ cm. in diameter and about 5 cm. from the bottom of the flask. The spore masses were found mostly within this circle, but a few were scattered in all directions about the opening; however, they were all included within a circle 4½ cm. in diameter concentric with the opening in the paper. The flask was then turned about so that the opposite side was exposed to the light through the opening in the paper. After 45 hours the paper was removed, and the same conditions as described above with regard to the arrangement of the spores about the opening were found to have been duplicated. In one experiment a grayish-black, instead of a jet-black, paper was used to exclude the light. In this case the spores, instead of being discharged towards the opening in the paper, were discharged against the side of the flask almost opposite the opening. This paper behind the glass acted as a mirror and it would seem that the reflected light exerted a stronger heliotropic influence than did the direct light. In this case the spore print covered an area on the side of the flask opposite and slightly below the source of light about six times larger than the area of the opening in the paper. The flask was then turned about, exposing the opposite side to the light, and the spores were again shot away from the direct light. However, when the paper was removed entirely the spores were discharged towards the window.

Buller shows that the great distance to which *Ascobolus immersus* spores are projected in comparison with that traveled by the spores of the Hymenomycetes is due to the large size of the spore mass. The size of this spore mass, he states, is due (1) to the unusually large size of the spores; (2) to the thick gelatinous envelope round each spore; (3) to the clinging together of the spores; and (4) to the large mass of the discharged ascus sap. In the case of *Pleurage curvicolla* there are approximately 500 spores all clinging together and discharged as one body together with a quantity of ascus sap or other gelatinous substance, making a large projectile. A number of spore prints made upon a microscopic slide placed over the mouth of the two-liter culture flask 26 cm. above the fruiting surface were measured. These were circular in outline and ranged from 168 to 266 μ in diameter. Surrounding each spore mass was a sort of halo about 25 to 30 μ wide due to the gelatinous substance in which the spores were imbedded and which was discharged along with them. No doubt the comparatively great mass of material discharged in this instance, as in the case of *Ascobolus immersus*, is a big factor in determining the distance to which the spore masses are shot.

Pleurage curvicolla probably can project its spores to a greater height than any other Ascomycete yet studied. Its spores are usually discharged in masses towards the source of light, but reflected light seems to exert a stronger heliotropic stimulus than does direct light.

CORRELATION BETWEEN SIZE OF THE FRUIT AND THE RESISTANCE OF THE TOMATO SKIN TO PUNCTURE AND ITS RELATION TO INFECTION WITH MACROSPORIUM TOMATO COOKE

J. Rosenbaum and Charles E. Sando

That artificial infection with *Macrosporium tomato* Cooke[1] from tomato on uninjured tomato fruit can be obtained, provided fruit of a certain maturity as measured by size is used, has been established by one of the writers.[2] The question naturally arises as to the cause of this apparent immunity or resistance in the fruit after it reaches a certain maturity.

Previous investigations along these lines have been recently reviewed and summarized by Hawkins and Harvey[3] as follows: "It is apparent that there is good evidence that some parasitic plants make their way into their host plants by breaking through the tissues mechanically. There is no doubt that some fungi secrete enzymes which break down the cell walls of certain plants and are thus able to make their way through the tissues of their hosts."

The same workers from their infection studies with Pythium on potato conclude: "There is considerable evidence that the main factor in this penetration is the growth pressure of the fungus filament, and the resistance of the white McCormick potatoes to this disease is due to cell walls that are more resistant to mechanical puncture than are the cell walls of extremely susceptible varieties."

The results obtained by Blackman and Welsford[4] in their studies with *Botrytis cinerea* on *Vicia Faba* are of special interest. They state that "the piercing of the cuticle is due solely to the mechanical pressure exerted by the germ tube as a whole or by the special outgrowth from it."

In the work reported in this paper the evidence obtained shows that:

1. While a chemical difference is found in the analysis of young and old fruits, this is not the limiting factor in infection with Macrosporium. The

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[1] The fungus causing typical "nail-head" spots on tomatoes has been shown in a paper which is being prepared for publication to be different from *Macrosporium solani* E. and M. For reasons given there this Macrosporium should be referred to as *Macrosporium tomato* Cooke.

[2] Rosenbaum, J. *Macrosporium solani* on tomato fruit (Abstr.). Phytopathology 9: 51. 1919.

[3] Hawkins, L. A. and Harvey, R. B. Physiological study of the parasitism of *Pythium debaryanum* Hesse on the potato tuber. Journ. Agr. Res. **18**: 275–297. 1919.

[4] Blackman, V. H., and Welsford, E. J. Studies in the physiology of parasitism. II. Infection by *Botrytis cinerea*. Annals of Botany **30**: 389–398. 1916.

fungus grows just as readily on the pulp and extracts of old fruits as on those obtained from young tomatoes. Moreover, positive infection has been obtained on fruits of all degrees of maturity when the skin is injured or removed previous to infection.

2. Surface sections of old and young fruits failed to reveal the presence of stomata or other natural openings in the skin.

3. As the tomato fruit develops, the surface of the fruit changes from a dull to a shiny appearance. The chemical nature of this change has not been determined, but sections cut from old and young tomatoes show that the cuticle increases in thickness with the age of the fruit. The development of the cuticular layer may be at least a partial explanation of the resistance of mature fruit to infection. Dewdrops are more readily retained on the dull surfaces than on the shiny and mature surfaces.

4. The maturity of the fruit as measured by size is correlated with a definite resistance of the tomato skin to puncture. The latter may also be one of the limiting factors in securing infection with Macrosporium on tomato fruit.

The methods employed in arriving at these conclusions and the detailed data obtained in this connection were as follows:

The tomatoes were grown in a commercial way in the fields of southern Florida. The work was limited to one variety, the "Livingston Globe." At first fruits of various sizes were selected at random. In order to get a more accurate knowledge of the fruit used, a large number of blossoms were tagged and pickings for stabbing and inoculations were made from these tagged blossoms at the end of each week. In this way it was possible to tell exactly the age of the fruit used, from blossoming time until the fruit began to show color in the field.

The Macrosporium cultures used in this work were isolated from tomato fruit. They were kept in pure culture and spores obtained according to the method described by Kunkel.[5] In a few cases spores were taken from fruit naturally infected in the field.

The resistance of the skin of the fruit to puncture was determined by the use of the Joly balance as modified by Hawkins and Harvey (*l. c.*). The construction of the Joly balance need not be given, but certain modifications of the balance and the methods followed in calculating the results obtained in the use of this apparatus in the present work will not be out of place here.

In using this apparatus for determining the resistance of the tomato skin, a fine glass needle 78 microns in diameter fixed to a glass rod with wax was suspended from the bottom of the pan. This needle was used throughout the experiments except in one instance as indicated. The needle and rod were well within the capacity of the spring of the balance. In operation the tomato was placed on the stand of the instrument; the needle was lowered until it just touched the surface of the tomato and watched closely until a quick drop showed that it had penetrated the skin of the fruit. The reading on the scale of the instrument was then taken. From this reading the pressure required to puncture the tomato skin could be calculated.

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[5] Kunkel, L. O. A method of obtaining abundant sporulation in cultures of *Macrosporium solani* E. & M. Brooklyn Botanic Garden Mem. **1**: 306–312. 1918.

To illustrate the method of calculation, let us suppose the scale reading at which penetration took place on a fruit to be 31.00. The upward pull of the stretched spring representing this number as the reading on the scale was determined by counterbalancing with weights placed on the pan. This pull equalled 7.42 grams. The entire weight of the glass rod, with the needle, was 12.04 grams. The pressure necessary to puncture the skin was, therefore, the difference between the downward force and the upward force, or the difference between 12.04 and 7.42, or 4.62 grams. The pressure necessary, therefore, to penetrate the skin of a particular fruit with a needle which was 78 microns in diameter amounts to 4.62 grams.

Where tomatoes of different sizes were used, a total of five fruits of each size were punctured. Since the hardness over the entire fruit varies somewhat, it was thought advisable to make part of the punctures around the style end and an equal number around the stem end. Generally ten punctures were made on each fruit. The average of these readings gives fairly accurately the pressure necessary to puncture a particular fruit. As a general rule, it was found that the stem end was slightly harder than the style end. The fruits were picked and brought into the laboratory where they were divided into two lots, each lot containing fruits of the same maturity. One lot was used for determining the resistance of the skin to puncture while the other lot was washed, placed in disinfected moist chambers, and inoculated by spraying with a suspension of Macrosporium spores. In addition to the inoculations made on fruits brought into the laboratory, additional inoculations were made in the field on fruits growing on the vines. In this case the fruit was sprayed with a suspension of spores and covered with a glazed paper bag for a few days. No difference in the amount of infection was obtained whether the fruit was inoculated in the

TABLE 1. *Showing the Relation Between Resistance of the Skin to Puncture and Macrosporium Infection on Different Sized Tomatoes* *

Size	Color	Circumference in Inches	Average Weight in Grams	Pressure in Grams Necessary to Puncture Fruit (Average of 50 Stabs)	Percentage of Positive Infection with Macrosporium
	Red	....	......	5.19	0
A....	Green	10 3/4–11 1/4	254.87	5.87	0
B....	Green	7 3/4– 8 1/4	115.12	5.70	0
C....	Green	6 1/2– 6 3/4	66.36	4.08	37 1/2
D....	Green	5– 5 1/2	34.22	3.52	85 5/7
E....	Green	4– 4 1/2	18.17	3.26	72 8/11
F....	Green	3– 3 1/2	7.39	2.66	100

* Temperature of tomatoes when punctured, 23° C.
Needle used, 78 microns in diameter.

laboratory or in the field. While a large number of punctures and inoculations were made throughout the season, the results are so uniform that it will suffice to present in tabular form a few representative series.

In Table 1 are shown the results of picking at random, at the same time, fruits of various sizes. These were divided into two equal lots, one of which was used for puncturing to determine the resistance of the skin, while the other was used for inoculations.

Examination of this table shows that the resistance of the skin to puncture increases with the size of the fruit, and likewise that the amount of infection varies from 100% in the case of the smallest fruit to 37½% in the case of fruit approximately 5–6 inches in circumference, with no infection above that size. From this series, the point at which the hardness of the skin begins to show any appreciable effect on infection is approximately that at which 4.08 grams of pressure is necessary to puncture the skin.

Table 2 shows the results of puncturing and inoculating fruits of known age for seven consecutive weeks. The fruits used in these series were all tagged when in blossom. The data show, as in the preceding table, that the older the tomato the more resistant is the skin, and that the amount of infection decreases when the resistance of the skin to puncture is approximately such that 5.08 grams of pressure is necessary to puncture the fruit with a 78-micron needle.

TABLE 2. *Showing the Relation Between Resistance of the Skin to Puncture and Macrosporium Infection on Tomatoes of Different Age*

Age in Days	Color	Weight in Grams, Average of 10 Fruits	Equatorial Diameter in Centimeters, Average of 10 Fruits	Temperature at which Stabbing was Done	Pressure in Grams Necessary to Puncture Fruit (Average of 100 Stabs)	Pereentage of Positive Infection with *Macrosporium tomato*
7*....	Green	0.24	0.70	..	0.97	100
14....	Green	6.74	2.30	21	2.99	100
21....	Green	64.66	5.18	25	4.21	85
28....	Green	82.37	5.40	22	4.90	49
35....	Green	95.10	5.46	21	5.08	23 1/3
41....	Green	147.91	6.55	21	5.96	0
48....	Green	91.86	6.92	23	6.74	0
55....	Turning	....	....	25	5.56	0
55....	Red	162.82	6.31	25	5.10	0

* Size of needle used for 7-day-old fruit, 46 microns in diameter. At all other ages punctured a 78-micron needle was used.

Tagging of blossoms to obtain fruits of a known maturity has shown that in the majority of cases age of fruit is a better indication of maturity than is size. As would naturally be expected, not all the tomatoes in a given lot of fruits attain the same size in a given length of time. Such fruits, however, have a resistance of the skin in proportion to their age rather than to their size, and react accordingly when inoculated. For this reason then, it seems, resistance of the skin to puncture is a better index of maturity than is the size of the fruit. The former is also preferable in predicting whether a certain tomato can or cannot be infected with Macrosporium.

SUMMARY.

In the development of a tomato fruit, the cuticular layer increases in thickness with the age of the fruit. Measurements to determine the resistance of the skin of tomatoes have shown that there is a definite and direct correlation between age and the resistance of the skin to puncture.

Infection experiments with *Macrosporium tomato* on tomato fruit have shown that the amount of infection which it is possible to obtain decreases with the age of the fruit.

While the results do not prove absolutely that the inhibition of infection is a purely mechanical one, the resistance of the tomato skin to puncture may explain, at least partially, the ease with which infection without previous injury is obtained on the young fruit but not on the older fruit.

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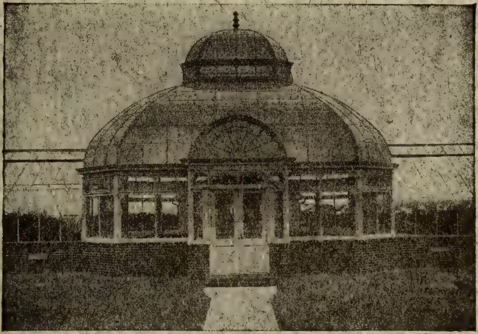

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THE LENGTH OF THE LIFE CYCLE OF A CLIMBING BAMBOO. A STRIKING CASE OF SEXUAL PERIODICITY IN CHUSQUEA ABIETIFOLIA GRISEB.*

WILLIAM SEIFRIZ

Certain plants are known to live vegetatively for many years, then flower and die. The most frequently cited example of this phenomenon is that of the century plant, *Agave americana,* which lives for a period of years without flowering, then sends up a tall, prominent inflorescence, and finally, after the maturing of the seeds, dies. This sexual periodicity is also characteristic of certain bamboos which blossom only after a cycle of years and then all simultaneously throughout an extensive region. The bamboos in the South Brazilian provinces of Santa Catharina and Rio Grande do Sul are said to blossom at intervals of about thirteen years, and *Bambusa arundinacea* on the west coast of Cisgangetic India blossoms at intervals of about thirty-two years (1). The complete and simultaneous dying off of the bamboos may in some communities prove disastrous by the wiping out of the chief available source of building material through the transformation of luxuriant bamboo forests into barren areas; or, it may prove of great economic value as a source of grain, especially when it comes, as it is said to (2), in times of drought and consequent famine.

The length of the interval of years varies greatly in different bamboos. Bean (3) reports that "*Bambusa tesselata* has been in cultivation for probably over sixty years, yet I have seen no record of its having flowered anywhere." In striking contrast with this is the case of *Arundinaria falcata* var. *glomerata* which flowers almost every year on a certain number of culms. The latter is a case of partial or sporadic flowering as contrasted with the complete and simultaneous flowering which is the rule among bamboos. Intermediate types also exist. Bean (3) mentions the case of *Arundinaria Simoni* which flowered on odd culms in the bamboo garden at Kew for several years. He says, "excepting that the flowering culms died, the plants were in no way affected. . . . They continued to flower in this way every year up to 1903, by which time we had almost come to regard *A. Simoni* as a perennial. In that year, however, the plants flowered on every culm, and, after producing an abundance of seed, died. After that

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* Botanical Contribution from the Johns Hopkins University No. 62.

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not a single trace of leaf growth was ever visible and the plants were ultimately uprooted."

This peculiar periodicity in the life of bamboos was strikingly brought to my attention during a recent stay in Jamaica. On my first walk along the trail which runs from Cinchona to Morce's Gap in the Blue Mountains, my attention was called, by Dr. Duncan S. Johnson, to the many dead patches of the climbing bamboo, *Chusquea abietifolia*. Dr. Johnson remarked that on three previous visits to Cinchona he had always found the Chusquea in full foliage, forming large entanglements which, like the tree ferns, stood out as a prominent feature of the tropical vegetation. The Chusquea was still there, interwoven into mats beside the mountain path or hanging in festoons above the trail, but the color was no longer green, for every plant seen on that first walk was dead. It was immediately suspected that this climbing bamboo had, true to the habits of its tribe, died as a result of profuse flowering following a long period of sexual inactivity. It seemed, therefore, advisable to collect all obtainable data bearing upon the life history of this Chusquea. These data here published will bring up to date the story of the life of the Jamaican Chusquea which was begun by Sir Joseph Hooker and Sir Daniel Morris thirty-three years ago. The present observations seem to fix the length of the life cycle.

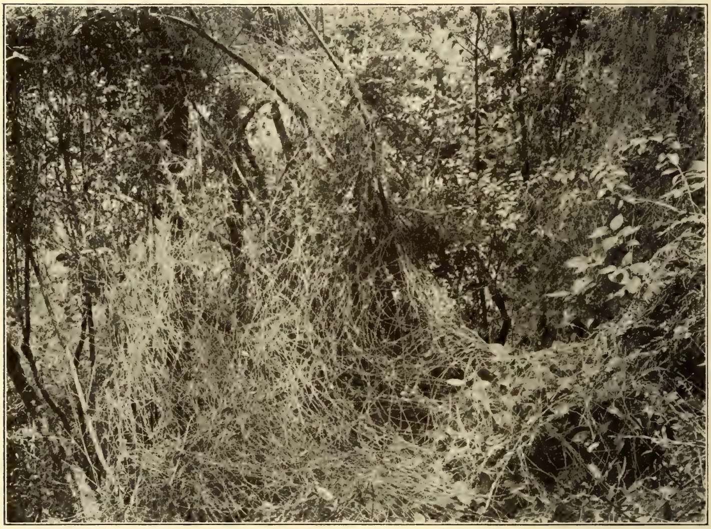

FIG. 1. An entanglement of dead *Chusquea abietifolia*.

The climbing bamboo, *Chusquea abietifolia*, is, in reality, a scrambler with no specialized climbing organs—although one's first encounter with the plant is likely to suggest the presence of vicious thorns, for the leaf midrib terminates in a very sharp, protruding point. The long, erect young shoots push upward among the surrounding plants and are held from slipping back by the subsequent development of whorls of leaves and lateral branches. The height attained seldom exceeds 30 feet, while the mats of interwoven stems are often 10 to 15 feet across. If high supports are lacking Chusquea succeeds very well in climbing over low shrubs. The maximum basal diameter of the old culms is hardly a quarter of an inch, in contrast to the 5- and 6-inch culms of the closely related *Bambos* (*Bambusa*) *vulgaris* of the Jamaican lowlands.

Chusquea abietifolia is little known outside of Jamaica. It has been reported (4) from only two other localities, both in the West Indies—Porto Rico (Monte Alegrillo) and Haiti (Monte Furcy). In Jamaica this rare bamboo is confined to the mountainous interior of the island. It does not occur much below 4,000 feet and is most abundant on the mountain ridges, being found on the very summit of Blue Mountain Peak, 7,360 feet above sea level.

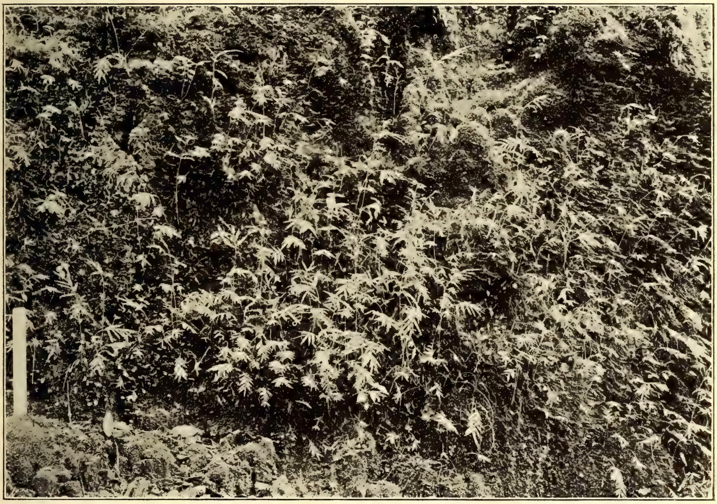

FIG. 2. Growing seedlings of *Chusquea abietifolia*.

The habitat of the plant is apparently not so definitely dependent on moisture as it is on altitude, although the lower limit of 4,000 feet is possibly fixed by moisture requirements. Rainfall in the higher altitudes of these tropical mountains is always ample for vegetation, yet there is a pronounced difference in the soil moisture of exposed ridges and shaded ravines. Chusquea is found in both these regions; on the sunny, hot, dry spurs where vegetation is relatively sparse, and in the dark, cool, wet gulches where tree ferns and other moisture-loving plants abound. Chusquea is, however, most abundant under conditions intermediate between these two.

Published descriptions of this climbing bamboo are few and brief, that of Grisebach, in his *Flora of the British West Indies*, being among the earliest. A more complete systematic account by J. D. Hooker appears in the *Botanical Magazine* (Curtis) for 1885. The first definite reference to sexual periodicity in Chusquea appears in a short notice by Morris in the *Gardener's Chronicle* for 1886. He writes, "The flowering of this plant appears to take place, as in most Bambuseae, at long intervals."

The data pertaining to the life habits of *Chusquea abietifolia* published here were obtained from the following sources: first, from Wm. Harris, government botanist of Jamaica, to whom I am greatly indebted for many kindnesses during my stay on the island: second, from several published articles herein referred to, which were kindly brought to my attention by Assistant Director Arthur W. Hill, of the Royal Botanic Gardens at Kew: third, from the notes of Daniel Morris and J. H. Hart recorded in a copy of Grisebach in the library of Hope Gardens, Jamaica: fourth, from the natives living in the mountains, especially David Watt, whose long experience in collecting for Jamaican and visiting botanists has made him uncommonly familiar with the plants of the mountain forests: and lastly, from my own observations covering a period of six weeks and extending over a ten-mile stretch of the Blue Mountain Range.

During June, 1919, nearly all mature plants of *Chusquea abietifolia* in the Blue Mountains of Jamaica were dead. On the other hand, the ground in many places was covered with seedlings varying from an inch to 18 inches in length. Diligent search brought to light only a few patches of old, living plants. Still fewer specimens were found bearing fruit.

The first question which naturally arose was, when did this climbing bamboo last flower? I was informed that there had just ended a most profuse flowering of all plants in the mountains wherever seen, and that the time of flowering extended over more than a year. The question which next presented itself was, how long a time had elapsed between this and the last previous flowering? Definite information on this point was obtained from Mr. Harris, who writes, "Chusquea flowered generally in the Blue Mountain regions in 1885–6 and died down everywhere." This first recorded flowering period also extended over more than a year, as is evident from the note of Hart supplementing that of Morris. The latter states that Chusquea was first noticed in flower in the fall of 1884, and that in

1885 it flowered generally. Hart adds, "It flowered also in 1886 or rather continued flowering from 1885."

That the outcome of the recent flowering of Chusquea—the death of all mature plants and their replacement by innumerable seedlings—is identical with that following the last general flowering 33 years ago, is evident from the writings of the early observers. Morris (5) tells the story in this manner: The Chusquea "began to shed its leaves and to assume a dull, rusty color . . . When the seed was set the stem began to die down, and apparently every plant in the island died, root and all. At the present time (1886) the ground in the forests where the Chusquea grew is covered with millions of seedlings, and in due time these will take the place of the former generation."

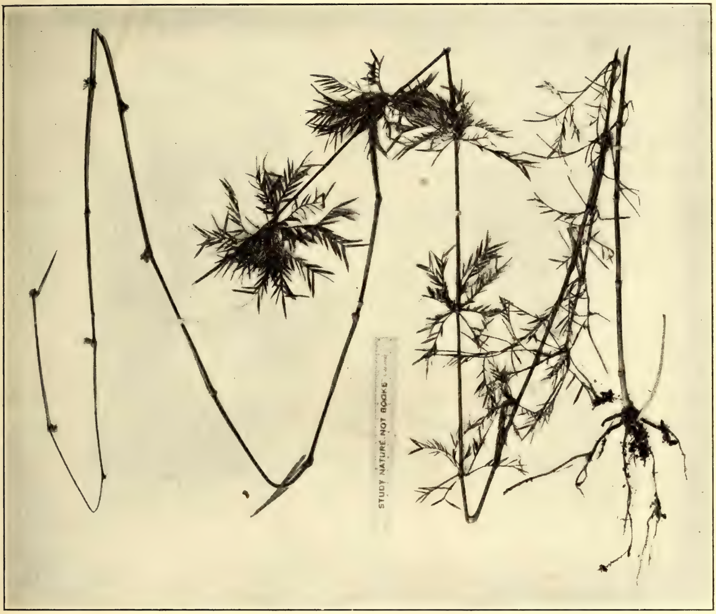

FIG. 3. An old basal culm of *Chusquea abietifolia* with a long, young, leafless shoot.

In 1884 some plants of Chusquea were sent in a Wardian case to the Royal Botanic Gardens at Kew. Hooker, referring to the Kew plants, wrote: "In December last (1884) they suddenly burst into flower causing me to fear that, after the manner of so many species of this most remarkable tribe of grasses to which they belong, they may not survive the flowering period." The Kew plants died just as did the wild ones. It is worthy of special note that the Kew plants, after being transplanted to an entirely new and different environment, flowered simultaneously with the wild plants in Jamaica.

It is, therefore, immediately apparent that *Chusquea abietifolia* had just ended (in 1918) a life cycle of about thirty-three years during which time it had grown vegetatively only, until the last year when it flowered, disseminated its seeds, and died.

There are but three other species of climbing bamboo, all belonging to the genus Arthrostylidium. These also, like the Jamaican Chusquea, are found only in the West Indies. It is very probable that at least one of these other species goes through a cycle similar to that of *Chusquea abietifolia. Arthrostylidium sarmentosum* has been collected in flower only once (6).

Many days of tramping over the mountain trails near Cinchona revealed but a single green specimen of Chusquea, the only living plant among many hundreds of dead ones bordering the trail in the two-mile walk from Cinchona to Morce's Gap. Whether the presence of this sole living mature plant among so many dead ones is due to certain edaphic conditions which have delayed flowering and thus possibly produced a plant of altered life cycle, is uncertain. Its possibility will be discussed in detail later.

The ascent of Blue Mountain Peak showed a similar state of affairs to exist in that locality. The trail to the summit was lined with innumerable patches of dead bamboo. Several green plants were found but these few were not fresh and thriving in appearance, being apparently in a dying condition.

Some days later I learned of green plants growing on an exposed, rocky spur. Investigation first revealed short, fresh, green tufts of Chusquea, which proved to be young shoots from old rootstocks. This region had recently been burnt over. The charred stubble was still evident. The presence of green Chusquea here seemed easily explainable: the parent plants had been burnt to the ground before their life cycle was complete, and the living rootstocks had sent up new shoots which were continuing the growth of the plants and thus carrying on the vegetative portion of the life cycle beyond the normal limit. Opposed to this supposition is the statement of Hackel (7) that small plants from cuttings or layers of bamboos blossom at the same time as do the parents from which they were taken. It would be very interesting to determine experimentally just how such a catastrophe as the destruction of that part of the plants above ground shortly before their time of flowering would affect the normal life history of a plant like Chusquea.

Continuing along the spur above mentioned, I subsequently found a fair-sized area with numerous old but green and thriving plants. They were not in flower but were healthy, actively growing specimens, sending out an abundance of long, young shoots. Here was a prominent exception to the general condition existing throughout the mountains. A noteworthy feature of the exception, however, was the fact that these healthy, green plants were all in a single and comparatively small area. The possibility of explaining their persistence by some external cause is, therefore, greater than would be the case had several distinct scattered groups been found.

The region in which these living plants are growing is one experiencing the extreme of mountain aridity above 4,000 feet. The ridge is hot and dry, and covered with vegetation characteristically xerophytic (*Pteris aquilina*, *Gleichenia Mathewsii*, *Agave americana*). Morce's Gap trail and Blue Mountain Peak, on the other hand, where Chusquea is, with few exceptions, to be found only as old, dead plants and young seedlings, are moist regions characterized by hygrophilous plants. Immediately below the dry area where the patch of living bamboos exists, there is a moist, shaded gulch where no living, mature Chusquea was found; for here, in an environment like that at Morce's Gap and on Blue Mountain Peak, the old bamboos are dead and seedlings are abundant. Here also flourishes a hygrophilous flora of tree ferns and succulent herbs. It seems, therefore, reasonable to conclude that the climbing bamboo has in this more arid region in some manner assumed an altered life cycle. The single green specimen, already referred to, found near Morce's Gap was growing on the hot and dry southwest slope of the mountain, a spot differing markedly from the nearby, shaded, semi-moist regions along the trail where Chusquea was represented by an abundance of dead plants and of living seedlings.

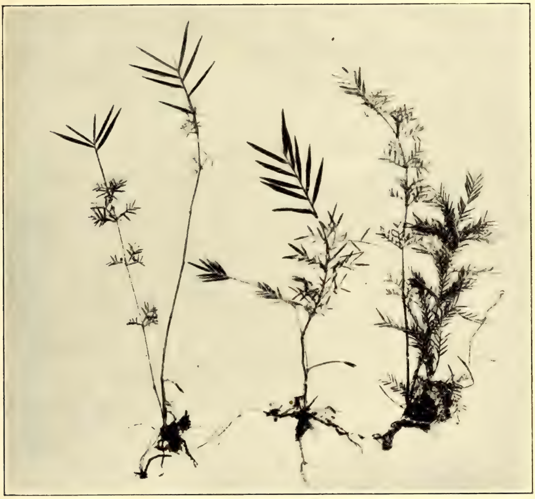
FIG. 4. Seedlings of *Chusquea abietifolia*.

One rather welcomes an exception to the striking regularity in sexual periodicity of a species extending over a large territory. Indeed, one would expect not a single exception but many, brought about by different rates in seed germination, and in growth both of seedlings and mature plants due to differences in environmental factors such as moisture, light, temperature, and soil, which would ultimately give rise to plants whose time of flowering would precede or follow that of the majority, and which would thus, in time, produce many plants whose life cycles overlapped so that some out of the many could be found in flower in any year. It would be exceedingly interesting to attempt to bring about such a state artificially by deferring the sowing of the seed, and thus attempting to postpone the time of flowering or to shorten the life cycle. It would seem, however, that this experiment must have been many times performed by nature (*i.e.*, if the seed is capable of germinating after 1 or more years) so that we should be able to judge from the present condition of the wild plants of Chusquea whether the cycle can be altered in this way. Bean (3) is of the opinion that the simultaneous flowering of bamboos follows some general law. What this general law might be he does not suggest. Yet he does believe that under cultivation the system of simultaneous flowering of some of these species would appear to be breaking down, and he cites the case of *Arundinaria Falconeri* which flowered in England, in the vast majority of cases, in 1876, but the flowering of the generation at the time he wrote (1907) had already extended over five seasons. That a breaking down of simultaneous flowering in *Chusquea abietifolia* is taking place in the wild state is suggested by the exceptions that I have noted and by the fact that this climbing bamboo was detected in flower in 1911 and was also flowering freely at the base of Catherine's Peak, but not elsewhere, in November, 1912. In fact, Mr. Harris suggests, "It is just possible that individual plants of Chusquea may be found in flower in any year if careful search were made for them."

In spite of these several exceptions, it remains a striking fact that fully 98 percent of all plants of *Chusquea abietifolia* found in Jamaica in a region some ten miles in length, varying from 4,000 to 7,000 feet in altitude, and showing considerable diversities of light, temperature, and moisture, have flowered and died in a single brief period not exceeding two years, after a purely vegetative growth of more than thirty-one years.

This complete cycle of thirty-three years differs by only one year from that given by Brandis (8) for *Bambusa arundinacea* in India. It seems quite possible that the life cycles of these two genera are the same, for the exact time of flowering is not always readily determined. The general flowering of a species in one particular year may be heralded by a few forerunners the previous year and followed by that of laggards the next. Morris states that the last previous flowering of Chusquea in the Blue Mountains of Jamaica commenced in 1884, and Hart reports it as continuing until 1886. The exact time of the recent flowering is not definitely known.

David Watt is of the opinion that he first saw Chusquea in flower in the fall of 1917. I myself collected a few fruiting branches in the early summer of 1919. The climbing bamboo was, therefore, probably at the height of its flowering period in 1885 and in 1918, making the cycle one of thirty-three years.

I have referred to the possible effect of environment on change in time of flowering. Equally interesting, and possibly as difficult of solution, is the ultimate cause of the simultaneous flowering of nearly all plants in a certain locality. The obvious suggestion, often made in such cases, that this peculiarity is innate, does not, of course, solve the problem. It simply indicates that we must seek our explanation in causes operative before the initiation of the individual.

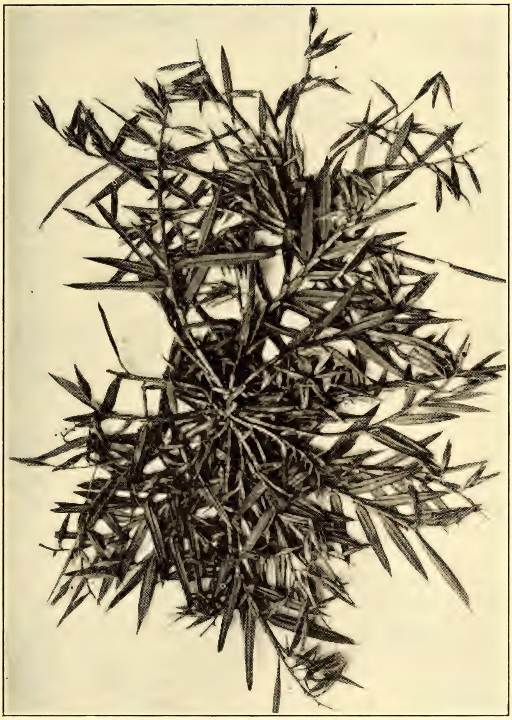

FIG. 5. Fruits of *Chusquea abietifolia*. Collector, William Harris.

Attempts to associate the sexual periodicity of plants with seasonal or other environmental changes may seem as far-fetched as to ascribe to climate or food the periodic appearance of the seventeen-year locust which has this year (1919) infested certain regions of our country. It is however, quite possible that the duration of the life cycle of plants exhibiting sexual periodicity is the direct result of certain known, present or past, stimuli. An apparently very clear example of such an association between season and periodicity is seen in the life cycle of annuals which flower and die at definite seasons of the year. But one can not always be certain that the most evident and seemingly controlling factor in such a case is the one at present active. A native annual in the temperate zone commonly rests in winter, germinates in the spring, fruits in summer, and dies in the fall. This sequence of events one is likely to attribute to the sequence of the seasons. Yet most annuals if grown in a greenhouse where seasonal changes are non-existent (except as to light) can, by sowing of seeds at the proper time, be made to fruit in any chosen month of the year without regard to seasonal conditions out of doors. Thus are the successive steps, from germination to death, in the life span of an annual grown in a greenhouse accurately maintained without evident relation to any external controlling factor. That is, the annual germinates, fruits, and dies in the same interval of time that it always has required, and does this in an environment quite different from the seasonally progressive one of its natural habitat. This behavior seems clearly to belie the validity of the assumption that the present seasonal round determines the duration of each phase of the developmental cycle, and thus of the cycle as a whole.

There are reported, however, examples of the flowering of plants being regularly brought on by such external factors as moisture. The following is such an example cited by Morris (5): "A prolonged drought in India is often accompanied by the flowering of the common bamboo, and on this account the natives associate the two phenomena in a manner which is emphasized by the fact that the bamboo grain during seasons of drought has provided them with the only available means of support." According to Ridley (cited by Schimper, 1) two species of Hopea and four species of Shorea blossom with great regularity every sixth year. These cycles are said to coincide with dry years. Morris (5) believes that the long intervals at which the flowering of Chusquea takes place probably depend "for their exact length upon the aspect of the prevailing seasons." Weather reports from Cinchona show, during the years preceding the recent flowering of Chusquea, no pronounced digression in temperature from the general average. The rainfall was unusually heavy for two years immediately preceding the flowering of Chusquea. It is hardly likely, though possible, that an over-abundance of rain should bring on a flowering period in Chusquea in Jamaica and drought be the cause of flowering of another bamboo in India.

Further proof against the theory that time of flowering is determined by present seasonal factors is to be had from the behavior of the climbing bamboos sent to Kew. Morris (5) himself presents a bit of evidence against his own contention when he says, "Both the wild plants at Jamaica and the cultivated plants at Kew (although the latter were under such very different conditions) were in flower at the same time."

Should the life cycle of *Chusquea abietifolia* prove not to vary from the thirty-three years which it has been found to be—just as the cycle of *Bambusa arundinacea* has, from three successive observations (1804, 1836, 1868) been found to be exactly thirty-two years (1)—then it would seem hardly likely that the length of this term of years could be definitely ascribed to present climatic influences, unless there is some larger climatic round of years, such as that suggested by Brückner (9).

It is interesting to note, though of how much significance this may be is pure conjecture, that the climatic oscillation ascertained by Brückner (9) closely approaches in length of years the life cycle of Chusquea. There is, however, in addition to Brückner's 35-year alternation of wet and dry epochs, a supposed variation of rainfall in a cycle of eleven years, coincidently with the sunspot cycle (10). It is as yet by no means well established that climatic changes are periodic, and there is but little to support the idea that droughts occur rhythmically, especially with any great precision.

In considering the possible relationship between sexual periodicity in plants and climatic oscillations I have had in mind—as I assume others have had in their attempts to associate the two—only present climatic influences. That past rhythmical variations in rainfall or in temperature have, through the ages, fixed the life-cycle of Chusquea is quite possible. So striking is the association between the life of an annual and the seasons that it seems very probable indeed that the cycle of annuals is the direct result of seasonal influences, and that this cycle has, through many generations, become so firmly established as to be unalterable through the temporary elimination of seasons by transferring the annual to a greenhouse.

This problem can, however, and should, be attacked from other viewpoints than the purely ecological one. Other factors than such external stimuli as droughts and similar seasonal epochs may have been at work in establishing, or still are at work in maintaining, sexual periodicity in Chusquea. The problem may be of the same nature as that of puberty and senility in organisms. We know, for example, that certain organisms require a certain number of years in which to reach sexual maturity, and we know that certain organisms live about so long and never exceed a certain maximum. The present causes of such phenomena are not as yet seriously thought to be environmental in nature. That they may be somewhat influenced by environment is possible. Lack of food, for example, is said to hasten the attaining of sexual maturity in man, but the digression from the mean is slight and has no direct bearing on the original establishment of the phenomenon.

In one respect, however, the periodicity of Chusquea differs strikingly from the cycle of annuals and the aging of organisms. That the span of life of an individual Chusquea is thirty-three years is no more remarkable and is as satisfactorily explainable as is the fact that an annual lives one year, man eighty years, and a Sequoia 5,000 years. But an hypothesis which will explain these phenomena is not necessarily sufficient to account for the simultaneous flowering of fully 98 percent of the individuals of a species extending over a great stretch of country.

There is as yet, it seems to me, no adequate explanation of the behavior of Chusquea. That seasonal factors at present active bring on this simultaneous flowering is very unlikely. That past climatic influences are responsible is quite possible. But the ultimate cause I should be inclined to search for in the physical and chemical make-up of its protoplasm; fully realizing, however, the possibility, indeed the probability, that this very nature of the protoplasm has come to be what it is in part because of its past environment as well as because of its own original constitution.

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Johns Hopkins University

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SEX INTERGRADATION IN THE FLOWERS OF MERCURIALIS ANNUA

Cecil Yampolsky

The older observers have repeatedly called attention to various aberrancies in plant structures, and these have been grouped under teratological phenomena. In this general category have been placed many unrelated phenomena as well as unexplainable ones. That has been particularly true with such occurrences as the appearance, upon a part of a plant which normally bore the organs of one sex, of the sex organs of opposite character. In another paper (1919) I have called attention to the fact that we may consider conditions such as the appearance of female flowers or branches on a male plant, male flowers or branches on a female plant, etc., as evidences of sex intergradation by no means uncommon in the plant kingdom.

In continuing my studies on *Mercurialis annua*, especially on the so-called monoecious form, I have observed some very interesting phenomena which appear to shed further light upon the question of sex determination. *Mercurialis annua* is described as appearing in three forms, male, female, and monoecious. The flowers of the female are, as a rule, two-carpeled, although often three-carpeled, and they are borne in clusters in the axils of the leaves. The flowers of the male are borne in interrupted spikes which surpass the leaves. The flowers of the so-called monoecious form (male, female, and hermaphroditic flowers) are borne like those of the female in clusters in the axils of the leaves.

The individual flowers of the three forms are minute and almost inconspicuous. The female flowers are apetalous, green—each carpel with a single ovule—a two-parted stigma which is white, translucent, and with roughened surface. In the instances in which there is either only one carpel or more than two, the stigma is undivided or multipartite depending upon the number of carpels. The carpels have a rough appearance due to the presence of characteristic translucent hairs. There are also two nectaries. The male flowers are apetalous with 8 to 20 stamens. Each stamen consists of a two-sacked yellow anther and a slender filament. The hermaphroditic flowers are like the female flowers with stamens borne from the bases of the carpels.

In a preceding paper (1919) I have reported upon the appearance of sporadic male flowers upon the female plants and of female flowers upon the male plants. Upon the female plants I have also noted and described various kinds of hermaphroditic flowers. Such flowers have also been observed on the so-called monoecious form but in much greater numbers.

Figures 1 to 7 of Plate V show a few of the forms that I have found. Figure 1 is a diagrammatic representation of a two-carpeled female flower showing (*s*) stigma, (*n*) nectary, and (*t*) hair. The appearance of the stigma and the hairs is particularly characteristic of the flower. Figure 7 is a diagrammatic representation of a male flower with several stamens, the dotted areas representing the anther sacs. In the female flowers there is never any evidence of an aborted or rudimentary stamen, nor is there any evidence in the male flower of an aborted or rudimentary pistil. Figure 2 is a diagrammatic representation of a very common type of hermaphroditic flower. The female elements are in all respects like those in the female flower. The male elements, the stamens, may arise at any point from the base of the carpel. The pollen grains are viable, and under favorable conditions self-pollination occurs and seed is set. In the light of what follows we may assume that the flower represented by figure 1 is more female than the flower represented by figure 2, in which the addition of a single stamen has added a characteristic of the male flower. Figure 3 shows a condition that is also quite common, an hermaphroditic flower with two stamens. These hermaphroditic flowers, too, are self-fertile, and seed is readily set. These flowers may be considered less female than the flowers represented by figures 1 and 2, inasmuch as there is an increase in the male elements. Figure 4 represents a condition in which more than two stamens occur together with the female elements. The number of stamens varies from three to sometimes more than eight. I have found many such flowers which have not been figured. Without sacrificing the functioning power of either the pollen grains or the ovules, various gradations in the proportion of male to female elements are produced in the hermaphroditic flowers. In figure 5 we have a condition in which the male elements have been substituted for half of the female elements. Figure 5 represents a flower with a single carpel, the other half of the flower being occupied by stamens. The number of stamens varies from 4 to 8. This arrangement differs from any described above because there has been a reduction in the amount of the female element so that, at best, the proportion of male and female elements is equal. In this condition, as in the preceding ones, there is no loss in the ability of the pollen or of the ovule to function either in self- or cross-pollination. Figure 6 shows a flower very similar to the one represented in figure 5 but with the addition of one or more stamens arising from the base of the single carpel. In this instance the male elements overbalance the female and the flower is decidedly more male than female. With the male and female flowers as the extremes of a series I have found, in my female and so-called monoecious cultures, intergrading flowers that suggest intergrading degrees of maleness and femaleness. It is interesting to note that this intergradation is not accompanied by sterility as is the case in the transition of ovaries into testes and testes into ovaries in the reported cases in animals.

In the three-carpeled flower I have not found the diversity of intergradation that I have described for the two-carpeled flower. Many of these three-carpeled flowers have been observed with a single stamen, some with two stamens, with three stamens, and with six stamens. The pollen when examined appeared to be perfectly healthy and normal.

While continuing the observations on the above described conditions within the flowers of the so-called monoecious form, an even more striking intergradation of parts, namely pistillody of the stamens and staminody of the pistils, was noted. There were hundreds of flowers that showed such a condition.

Botanical literature abounds with many illustrations of the transmutation of pistils into stamens and of stamens into pistils. The texts on teratology give many illustrations. Without going further into the literature on this subject, I wish to cite the work of Haring (1894). He gives an elaborate series of drawings showing various transition stages of stamens into pistils and of pistils into stamens in *Salix caprea* L. and *Salix cinerea* L. To Haring this facility with which one sex organ is transmuted into another is an indication of the morphological equivalence of sex organs of the opposite sexes.

In the flowers of Mercurialis under observation, most elaborate and varied transition stages appeared of stamens into pistils and of pistils into stamens. Because of the minuteness of the flower, the flower buds were removed and examined under a binocular microscope. Figure 8 represents a female flower with an anther sac growing from one side. As far as could be determined, the ovaries were normal. The pollen grains were for the most part plump and appeared healthy. Figures 9 and 10 are parts of a single three-carpeled hermaphroditic flower. The carpels appeared to be normal; the stamens showed transition stages into pistils. In one stamen (fig. 10) there was one anther sac, in a second one (fig. 9) one of the anther sacs was much reduced in size. There can be no doubt that these stamens had been partially transformed into female tissue, because they showed the white translucent stigmatic surface and the hairs, characteristic of the female flower. The anther sacs were yellow like those of the normal stamens. The pollen from each of the anthers appeared healthy.

Figures 11, 12, and 13 are parts of a two-carpeled hermaphroditic flower with what appear to be two anthers. Figure 11 is evidently a transmuted stamen, showing the stigmatic tissue and the hairs of the female flower. There was no trace of an anther sac or of an ovary. It was completely sterile. The second stamen (fig. 13) had the characteristics of both the stamen and the pistil. It had two anther sacs of unequal size and the stigmatic character and hairs of the pistillate part of the flower. One of the carpels (fig. 12) had imbedded within its tissue an anther sac. The majority of the pollen grains in this sac were plump and appeared normal. This flower, then, showed both staminody of the pistils and pistillody of the stamens in a very marked degree.

Figures 15 to 19 are of a single three-carpeled hermaphroditic flower with four stamens. In only one of the stamens (fig. 15) was there an anther sac. The pollen grains were for the most part plump and appeared healthy. The other three anthers (figs. 16, 17, 18) looked externally like individual carpels although internally there was no evidence of differentiation into fertile and sterile tissue. The three carpels (fig. 19) contained differentiated ovules somewhat enlarged in size and they had an appearance that suggested oedema.

Figures 20 to 22 represent parts of a three-carpeled female flower almost entirely transmuted into stamens. The fact that the flower contained only three parts is evidence that it is a three-carpeled female flower and not a reduced male flower. The pollen grains from each of the sacs showed few shriveled grains.

The male flowers show an equally strong tendency to grade into the female through a series of gradations, from the faintest suggestion of a stigma to the presence of an ovary and of an apparently normal ovule. Figure 24 shows a male flower with ten stamens, two of which have been partially transformed into female tissue, each bearing one anther sac. The remaining eight stamens are normal. Most of the pollen grains in the sacs of the transmuted stamens were normal in appearance.

Figures 25 to 30 represent parts of a single male flower, seven of which were normal, the rest in various stages of transformation. Figure 30 is a normal stamen. Figure 27 represents a stamen with an indication of a stigma, the tissue being white and translucent. The stamen in figure 25 is a little more female, while figures 26 and 29 show more extreme conditions of femaleness. Figure 28 shows a combination of male and female elements, a fully developed stamen and a carpel with a well-developed ovule.

Figure 14 is a part of a male flower much like the one described above, but in which only two stamens were abnormal. The stamen represented by figure 14 had a completely developed ovary (*o*) and two large anther sacs with apparently healthy pollen.

Figure 23 shows a stamen with four anther sacs and a completely developed ovary. This was taken from a flower otherwise male.

It is impossible here to give the numberless variations that occurred in all three kinds of flowers. The illustrations cited give an idea of what is occurring. This evidence of pistillody of the stamens and of staminody of the pistils, coupled with the apparently normal arrangements of male and female elements as seen in figures 1 to 7, emphasize all the more strongly that intergradations within the flowers may occur by steps that are almost insensible. With the female flower as one extreme and the male flower as the other, flowers may grade all the way toward femaleness from the male extreme and all the way toward maleness from the female extreme.

While it has not yet been determined whether or not intergradation is here accompanied by sterility, the indications from the appearance of the pollen and the ovules suggest that total sterility does not occur. Goldschmidt (1916), in his crosses between European and Japanese races of the gypsy moth, *Lymantria dispar*, secured individuals that showed gradations in maleness and femaleness. Such a condition was usually accompanied by sterility. Banta (1916, 1918) finds that in his Crustaceans the more pronounced the sex intergradation the more sterile the form became. While a flower of a plant may not be compared to an individual like a moth or a Crustacean, it is interesting to note that in plants sex intergradation is not, as a rule, accompanied by sterility. On the contrary, the plants whose flowers were studied showed no diminution in seed production or in general vigor.

The condition of intergradation within a series of flowers on a single plant brings up interesting considerations bearing upon the question of when and how the sex of each flower is determined. The *Anlagen* or determiners, if there be such, must be different for the different flowers according to the arrangement of their parts. It must be borne in mind that in this so-called monoecious form of Mercurialis the sex of the plant changes in the course of the plant's development. The initial flowers are female. Several weeks after germination the young plant produces female flowers, and only female flowers are produced for several months. Then a few male flowers or hermaphroditic flowers appear. These increase in number as the season advances. As far as the whole plant is concerned, there is a periodic alteration of sex. A factorial hypothesis for sex cannot explain these results. It would seem logical to assume that the sex of the flower is determined at the time of its formation and not when the plant of which it is a part is in the fertilized egg stage. Moreover what seems to hold true for such flowers as represented in figure 1 to 7 apparently does not hold for the aberrant conditions I have noted. In the various transitional forms there seems to be no definite factor which determines the sex of the flower; pistil passes into stamen and stamen into pistil at any time in its development. The argument for strict sex segregation is obviously nullified because of the behavior of these forms. The line of demarcation between what is male and what is female is wavering and vague. The evidence brought out here tends to emphasize an epigenetic condition for sex rather than the presence of definitely localized qualitative or quantitative factors.

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EXPLANATION OF PLATE V

FIG. 1. Female flower: (*s*) stigma, (*n*) nectary, (*t*) hairs.

FIG. 2. Hermaphroditic flower with one stamen.

FIG. 3. Hermaphroditic flower with two stamens.

FIG. 4. Hermaphroditic flower with more than two stamens.

FIG. 5. Hermaphroditic flower with single carpel, the other half occupied by stamens.

FIG. 6. Hermaphroditic flower with single carpel, the other half occupied by stamens, also two stamens from the base of the single carpel.

FIG. 7. Male flower.

FIG. 8. Female flower with a single anther sac without filament budding out of the side of a carpel.

FIGS. 9–10. Parts of a three-carpeled hermaphroditic flower, the three stamens showing transitional stages.

FIGS. 11–13. Parts of a two-carpeled hermaphroditic flower. FIG. 11. Stamen completely changed into sterile tissue with female characteristics. FIG. 12. Anther sac imbedded within the tissue of one carpel.

FIG. 14. Part of a male flower having anther sacs and (*o*) ovule.

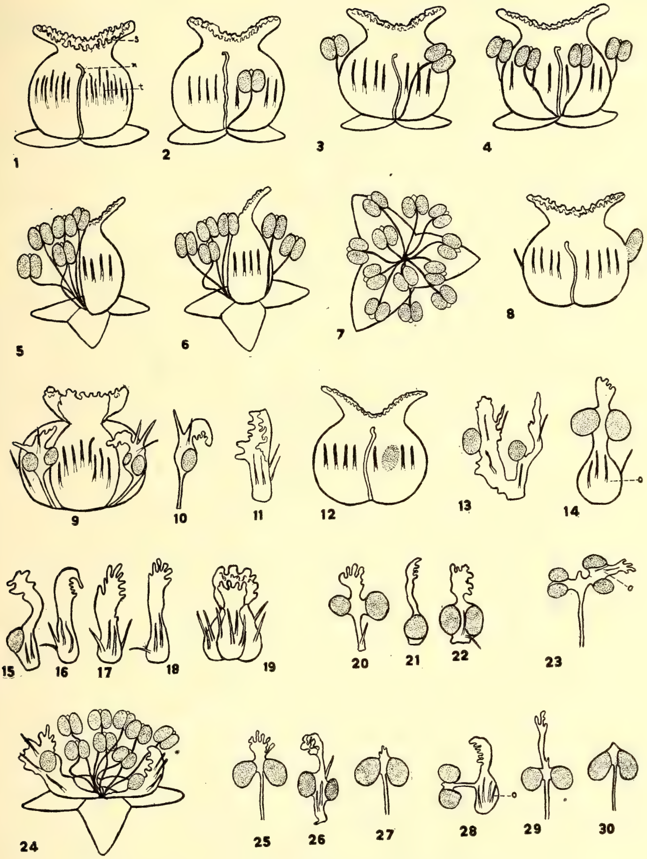
FIGS. 15–19. Parts of a three-carpeled hermaphroditic flower. Figs. 15–18. Transformed stamens: (*as*) anther sacs.

FIGS. 20–22. Parts of a three-carpeled female flower transformed into stamens.

FIG. 23. Stamen with four anther sacs and a single ovule (*o*).

FIG. 24. Male flower with two stamens in transitional stages.

FIGS. 25–30. Parts of a male flower in various transitional stages: (*o*) ovule, (*ns*) normal stamen.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YAMPOLSKY: SEX INTERGRADATION IN MERCURIALIS ANNUA.

THE UPWARD TRANSLOCATION OF FOODS IN WOODY PLANTS. I. TISSUES CONCERNED IN TRANSLOCATION

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It seems to be very generally believed that in shrubs and trees there is storage of organic matter in the lower part of the trunk and in the roots, and that, as growth starts in the spring, this food becomes soluble and passes upward through the xylem to the developing shoots and leaves.

The fact that has been most commonly offered as proof of upward translocation through the xylem is that the xylem tissues of woody plants commonly contain quantities of organic storage products, particularly sugars and starches. It has been shown by Atkins (1916), Fischer (1891), and others that not only the living parenchyma cells but also the water-conducting cells proper, the tracheae and tracheids, contain appreciable quantities of soluble carbohydrates, and Atkins has found these to be present at all seasons of the year. The latter considers that sugars are commonly carried through the xylem. He even goes so far as to say that ". . . the transference of carbohydrates can no longer be regarded as an occasional and accessory function of the vessels, but is certainly a continual and principal function . . . ." It may well be, however, that there is no flow of solution through the xylem for any great distance, for a possible frequent interposition of living cells across the water-conducting tissues may prevent such a flow. Atkins himself clearly recognizes that water may flow across tissues containing a high solute content without carrying the solutes with it.

Experiments performed by Hartig (1858) are also commonly cited in texts as offering proof of upward translocation through the xylem. Hartig ringed a number of trees early in the summer and found early in the following spring that the starch stored below the ring had disappeared. He concluded that the storage products must have been translocated upward through the xylem. It might be, however, that the food stored below the ring was used locally for growth in diameter, or that it was translocated downward through the phloem and was used in root growth.

In order to determine more definitely whether the upward translocation of food takes place primarily through the phloem or through the xylem, the writer has conducted a series of experiments part of which are reported in this paper. Some of these experiments also throw light on the matter of food movement from roots or trunks to growing shoots. The discussion therefore naturally falls into two main parts: (1) When upward translocation of organic matter takes place, as it certainly must for at least short distances, does it take place through the water-conducting tissues, the xylem, or through the phloem? And (2) Is there normally an upward movement of this food from the roots and lower trunk to the growing shoot? The present discussion will deal with the former question; the second question will be discussed in a subsequent paper.

EFFECT OF RINGING ON GROWTH OF LEAFY AND DEFOLIATED SHOOTS.

Vigorous young shoots of *Philadelphus pubescens* Loisel. were treated on May 30 as indicated in table 1. In this as in subsequent tables those shoots having the same letter were fairly well matched as to size, vigor, and position on parent plant.

TABLE 1. *Philadelphus pubescens. Rings in new growth 15–20 cm. from tip. Stems measured from attachment to main stem. May 30 to June 8, 1918*

	1 Not Ringed. Leaves Remaining			2 Ringed 15–20 Cm. from Tip. Leaves Remaining			3 Not Ringed. Leaves Removed from Upper 15–20 Cm.			4 Ringed 15–20 Cm. from Tip. Leaves Above Ring Removed		
	Length in Cm. May 30	Length in Cm. June 8	Gain in Cm.	Length in Cm. May 30	Length in Cm. June 8	Gain in Cm.	Length in Cm. May 30	Length in Cm. June 8	Gain in Cm.	Length in Cm. May 30	Length in Cm. June 8	Gain in Cm.
a . . .	52.5	81.5	28.5	61.0	79.0	18.0	40.0	69.0	29.0	58.5	58.5	0.0
b. . . .	58.0	79.5	21.5	60.0	75.0	15.0	53.5	85.0	31.5	60.5	61.5	1.0
c. . . .	49.5	85.0	35.5	78.0	Broken	—	40.5	66.0	25.5	63.5	81.5	18.0[1]
d. . . .	54.0	90.0	45.0	61.0	67.0	6.0	64.0	92.5	28.5	55.0	55.5	0.5
e	59.0	99.0	40.0	59.0	67.0	8.0	60.0	80.5	20.5	58.0	80.0	22.0[1]
Average gain			34.1			11.75			27.0			0.5

The measurements of June 8 show very clearly that ringing has in some way checked the growth of the shoots. The simplest explanation appears to be that the food necessary for shoot formation passes upward through the phloem and not through the xylem tissues. The few leaves present above the ring (group 2) are able to synthesize sufficient food to allow for some growth. In two shoots of group 4 a narrow strip of phloem was left by accident, and this has served to transfer sufficient food to allow for considerable growth. Two or three small leaves unfolded at the apex of the stems of group 4, but these were too small to carry on much photosynthesis, so growth continued very slowly for several weeks until other leaves had developed.

The same experiment was repeated with Philadelphus in the spring of 1919, except that in this case, instead of making the ring in the upper part of the new growth, the ringing was done on the wood of the previous year just below the bases of the young shoots. As the stems had been cut back when dormant, this left the new shoots at the apex of the stem. In each case, whether the leaves were or were not removed, one pair of leaves enclosing the growing tip was removed. The measurements are recorded in table 2, and a photograph of those shoots lettered *g* is shown in figure 1.

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[1] Not included in average. A strip of phloem was left on about ¼ of the circumference. The twig was bent at an angle at this point.

TABLE 2. *Philadelphus pubescens. Paired shoots measured from attachment to old stem. Rings made on old wood just below attachment of young shoots. May and June, 1919*

	1 Not Ringed. Leaves Remaining			2 Ringed. Leaves Remaining			3 Not Ringed. Leaves Removed			4 Ringed. Leaves Removed		
	Length in Cm. May 30	Length in Cm. June 4	Gain in Cm.	Length in Cm. May 30	Length in Cm. June 4	Gain in Cm.	Length in Mm. May 30	Length in Mm. June 4	Gain in Mm.	Length in Mm. May 30	Length in Mm. June 4	Gain in Mm.
	19.0	35.5	17.5	16.0	27.5	11.5	4.0	10.5	6.5	14.0	15.5	1.5
a.....	18.5	33.5	15.0	14.5	26.5	12.0	16.0	27.5	11.5	12.5	14.0	1.5
	17.0	30.5	13.5	15.0	27.5	12.5	13.0	20.5	7.5	19.0	21.5	2.5
b.....	17.5	31.5	14.0	18.5	31.5	13.0	14.5	22.5	8.0	16.5	17.5	1.0
	25.5	46.5	21.0	21.0	29.0	8.0	19.5	26.0	7.5	15.0	16.0	1.0
c.....	13.0	30.0	17.0	23.0	35.0	12.0	18.0	22.5	4.5	19.0	20.5	1.5
	23.0	40.5	17.5	20.5	35.0	14.5	17.0	25.0	8.0	16.5	15.5	2.0
d.....	23.0	42.0	19.0	24.0	38.0	14.0	15.0	24.0	9.0	20.0	21.5	1.5
	12.5	28.0	15.5	24.0	39.5	15.5	16.0	25.0	9.0	15.5	17.0	1.5
e.....	18.5	35.0	16.5	24.5	40.0	15.5	23.0	35.5	12.5	15.5	17.0	1.5
	20.0	36.5	16.5	21.5	33.5	12.0	16.5	19.0	2.5	20.0	22.5	2.5
f.....	20.0	38.5	18.5	21.5	35.0	13.5	24.5	28.0	3.5	20.5	22.5	2.0
	15.5	29.0	13.5	24.5	38.0	13.5	24.5	30.0	5.5	28.5	30.0	1.5
g.....	20.5	34.5	14.0	27.5	41.5	14.0	21.5	27.5	6.0	26.5	27.5	1.0
Ave.			16.36			12.96			7.96			1.61
	Length in Mm. June 3	Length in Mm. June 6	Gain in Mm.	Length in Mm. June 3	Length in Mm. June 6	Gain in Mm.	Length in Mm. June 3	Length in Mm. June 6	Gain in Mm.	Length in Mm. June 3	Length in Mm, June 6	Gain in Mm.
	32.0	39.5	7.5	38.0	45.0	7.0	45.0	47.5	2.5	24.0	25.5	1.5
h.....	27.0	35.0	8.0	33.0	40.0	7.0	43.0	46.5	3.5	38.0	38.5	0.5
	28.0	36.0	8.0	41.5	49.0	7.5	36.5	40.0	3.5	36.0	37.5	1.5
i.....	43.0	53.5	10.5	38.0	45.5	7.5	26.5	29.5	3.0	25.5	27.5	2.0
	42.5	54.0	11.5	42.5	51.0	8.5	44.5	48.0	3.5	35.0	36.5	1.5
k.....	42.5	52.5	10.0	31.5	38.5	7.0	37.0	40.0	3.0	30.5	32.0	1.5
	42.5	52.5	10.0	40.5	47.0	6.5	46.0	50.5	4.0	28.5	30.0	1.5
l.....	43.5	53.0	9.5	42.0	48.0	6.0	41.5	46.5	5.0	28.5	30.0	1.5
Ave.			9.38			7.13			3.56			1.44

The results of this second experiment are similar to those of the previous one. It is noticeable, however, that the ringed shoots with leaves (group 2) in this experiment show greater growth than shoots without leaves and not ringed (group 3), while in the previous experiment the reverse was true. In the experiment of 1918 only a third to a half of the new shoot was above the ring or was leafless, while in that of 1919 the whole of each new shoot was above the ring. It seems very probable that the older leaves of the new shoot manufacture a large part of the food used in terminal growth after the shoot is well started.

A similar experiment was tried with a tree of Northern Spy apple. This was started near the end of the growing season, which fact accounts for the small amount of growth obtained. All growth on this tree was practically completed by June 30, whereas with most of the other trees of the orchard it was nearly completed on June 11.[2]

[2] The writer is much indebted to Prof. W. H. Chandler, of the department of pomology, who very kindly offered him the trees of the department orchard for experimentation, and to Prof. E. C. Auchter, now of the Maryland Agricultural Experiment Station, who assisted in these experiments with the apple.

TABLE 3. *Pyrus malus* (*Northern Spy*). *Rings made 15 cm. from tip in present year's growth. Measurements from last bud scale scar to tip. June 11 to June 30, 1918. All growth was practically completed by June 30*

	1 Not Ringed. Leaves Remaining			2 Ringed. Leaves Remaining			3 Not Ringed. Leaves Removed			4 Ringed. Leaves Removed		
	Length in Cm. June 11	Length in Cm. June 30	Gain in Cm.	Length in Cm. June 11	Length in Cm. June 30	Gain in Cm.	Length in Cm. June 11	Length in Cm. June 30	Gain in Cm.	Length in Cm. June 11	Length in Cm. June 30	Gain in Cm.
a....	26.2	30.5	4.3	24.5	30.0	5.5	23.0	25.5	2.5	23.3	23.0	0.0
b....	26.0	29.5	3.5	17.5	22.0	4.5	24.8	28.0	3.2	21.5	21.0	0.0
c....	26.5	32.5	6.0	23.5	Broken	—	19.5	23.5	4.5	24.5	25.0	0.5
d....	25.0	29.5	4.5	20.0	24.5	4.5	23.3	28.0	4.7	22.5	23.0	0.5
e....	20.7	22.0	1.3	26.5	32.5	6.0	24.0	24.5	0.5	22.4	24.5	0.1
f....	25.7	33.0	7.3	23.0	29.0	6.0	22.2	27.0	4.8	24.6	25.0	0.4
Ave.			4.48			5.3			3.37			0.25

It is to be noted in this case that the ringed twigs which retained their leaves made a growth greater than did similar twigs not ringed. This difference is not striking, but in only one pair (*f*) is there greater growth of the stem not ringed. It would seem that food manufactured by the leaves was beginning to be removed downward and that the ring had checked this removal, thus increasing the supply for continued apical growth. The final cessation of growth of all twigs is evidently brought about by conditions correlated with the rest period which will not be discussed here. The almost complete lack of growth of the ringed twigs that had been deprived of their leaves coincides with the similar results obtained with Philadelphus. Evidently ringing has entirely prevented the upward translocation of some material or materials necessary for growth.

Further experiments of the same nature were tried with *Ligustrum ovalifolium* Hassk. Sone of the results obtained are presented in table 4. Other experiments showed results practically identical with these, so it is hardly necessary to present all of them.

From these experiments it seems very clear that the ringing has prevented the upward movement of some substance or substances necessary for growth. When leaves are present above a ring, these produce enough of this substance to allow for continued growth. Evidently the check in growth is not due to lack of water resulting from injury to the xylem, for those stems that were ringed but retained their leaves always made fair growth, which occasionally even exceeded that of the checks. Under certain conditions, however, ringing does seem to be followed by withering of the parts above the ring. This occurs especially when the ring is fairly near the young tip but only when no leaves are left above the ring. Hanstein (1860) obtained similar results and concluded that lack of water cannot be the cause of the withering, for when leaves remain above the ring, certainly more water is necessary but growth continues, while the shorter the part above the ring the less water it will need but the quicker is its death, even in a moist atmosphere.

TABLE 4. *Ligustrum ovalifolium. Bushes killed nearly to the ground by the cold winter. Experiments with the strong young shoots, June, 1918. All ring wounds had healed over by August 1. Some of no. 4, however, were not perfectly healed*

Hedge No. 1	1 Not Ringed. Leaves Remaining		2 Ringed 20–24 Cm. from Top. Leaves Remaining		3 Not Ringed. Leaves Removed for a Distance of 20–24 Cm. from Top		4 Ringed 20–24 Cm. from Top. Leaves Above Ring Removed	
	Growth in Cm. June 18–July 3	Growth in Cm. July 3–Aug. 1	Growth in Cm. June 18–July 3	Growth in Cm. July 3–Aug. 1	Growth in Cm. June 18–July 3	Growth in Cm. July 3–Aug. 1	Growth in Cm. June 18–July 3	Growth in Cm. July 3–Aug. 1
a.......	15.0	33.5	15.0	23.5	7.5	26.5	0.5	14.5
b.......	15.0	32.5	16.0	29.5	9.5	34.5	1.0	12.0
c.......	13.8	31.5	12.3	20.5	8.0	28.0	1.0	10.0
d.......	10.5	27.5	14.5	27.5	7.5	21.0	1.0	15.5
e.......	9.0	20.0	15.0	20.0	5.5	16.0	0.5	12.0
f.......	15.0	25.0	15.5	broken	6.5	10.5	0.5	9.5
g.......	17.0	32.5	14.0	27.0			0.5	13.0
Ave.....	13.61	28.93	14.61	24.67	7.41	22.75	0.71	12.36
Hedge No. 2	Growth in Cm. June 19–July 3	Growth in Cm. July 3–Aug. 1	Growth in Cm. June 19–July 3	Growth in Cm. July 3–Aug. 1	Growth in Cm. June 19–July 3	Growth in Cm. July 3–Aug. 1	Growth in Cm. June 19–July 3	Growth in Cm. July 3–Aug. 1
h.......	15.0	30.0	15.0	13.0	3.5	25.0	0.4	16.1
i.......	11.0	18.5	10.0	11.5	5.5	19.5	0.5	1.0
j.......	13.5	17.0	11.5	12.0	8.0	15.0	0.0	13.0
k.......	12.5	26.0	9.0	broken	7.0	12.0	0.0	5.5
l.......	10.0	29.5	9.5	9.0	6.5	18.0	0.0	2.2
m......	12.5	22.5	8.5	3.0	4.0	broken	0.5	5.0
Ave.....	12.49	22.41	10.58	9.7	5.75	17.9	0.23	7.11

Hanstein explained this lack of growth and the death above a ring when the leaves are removed on the grounds that "newly assimilated sap" is necessary. He accepted Hartig's idea that the stored food, particularly carbohydrates, readily moves upward through the xylem, but believed that this newly assimilated food moves in the phloem only. When leaves remain above a ring, they supply this essential food. Furthermore, Hanstein found that ringed willow cuttings placed in dry air showed a withering of the phloem above the ring while the presence of leaves prevented withering. He concluded that water cannot readily move from xylem to phloem and that the leaves aid in this transfer. According to his ideas, therefore, the leaves supply "newly assimilated sap "which is necessary for growth and can be carried through the phloem only, and they aid in transmitting the water to the phloem when the latter is separated from the roots by a ring.

If, however, all foods, including sugars, were translocated upward through the phloem only and not through the xylem, ringing would check further growth by withholding all necessary foods, and the withering might be due therefore not to the lack of any particular food, newly assimilated or otherwise, but to a deficiency of osmotically active substances, perhaps carbohydrates. Chandler (1914) has clearly demonstrated that, if tissues having different osmotic concentrations are organically connected, that tissue having the higher concentration will withdraw water from the other, causing the latter to wither. He demonstrated that leaves may withdraw water from fruits, or that a tomato plant having a high sugar content, if grafted to one with a lower sugar content, may withdraw water from the latter, causing it to wither.

In order to determine whether ringing and defoliation have any effect on the concentration of the sap, the freezing points of the sap from some of the stems of Philadelphus, previously described in table 2, were determined. The shoots were in pairs, as shown in figure 1, and for each determination both stems of the pair were used. As soon as possible after cutting the shoots, the leaves were removed and the stems were immediately cut into short pieces and placed in large test tubes which were then quickly plunged into a freezing mixture. After the tissues were thoroughly frozen they were ground in a mortar and the freezing point of the pulpy mass was determined. The material from each of numbers 1, 3, and 4 was extracted in 80 percent alcohol and this extract was hydrolyzed and analyzed for reducing sugars. The total sugar found, expressed as invert sugar, is recorded with the other

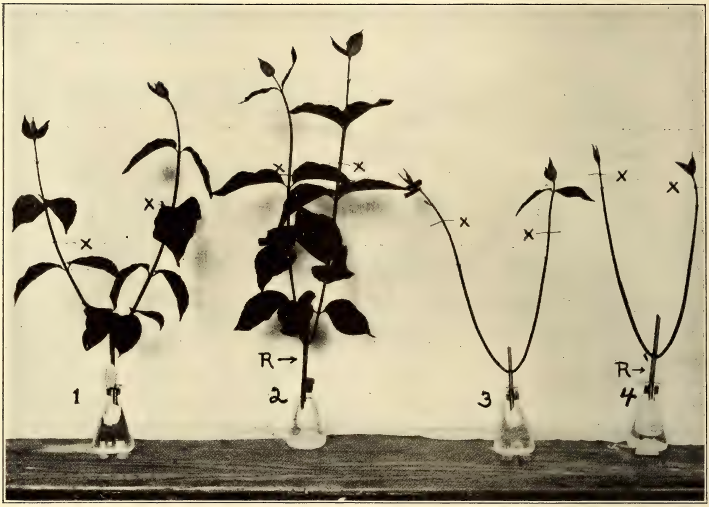

FIG. 1. Effects of ringing on leafy and defoliated shoots of Philadelphus.
1. Not ringed, leaves remaining.
2. Ringed, leaves remaining.
3. Not ringed, leaves removed.
4. Ringed, leaves removed.
Black strings tied at *x* indicate the original length of the shoots.

data in table 5. In table 6 are given the freezing point lowerings of the terminal pair as well as of the first pair of stems below the terminal pair. These were determined for the same series but on a different day.

TABLE 5. *Philadelphus pubescens. Effect of ringing and removal of leaves on growth osmotic concentration, and soluble sugar content of the stem*

		Length of Shoots in Cm. May 30	Length of Shoots in Cm. June 4	Gain in Cm. Length	Ave. Gain per Pair	Freezing Point Depression Δ	Green Weight	Total Dry Weight	Mg. Invert Sugar per Gm. Green Weight	Mg. Invert Sugar per Gm. Dry Weight
1. Not ringed, leaves present	*a*	19.0	36.5	17.5			10.0			
		18.5	33.5	15.0	16.25	0.65°				
	d	23.0	40.5	17.5						
		23.0	42.0	19.0	18.75	0.69°	15.6	1.74	7.81	114.9
2. Ringed, leaves present	*a*	16.0	27.5	11.5						
		14.5	26.5	12.0	11.75	0.60°	8.9			
	d	20.5	35.0	14.5				—	—	—
		24.0	38.0	14.0	14.25	0.62°	12.0			
3. Not ringed, leaves removed	*a*	4.0	10.5	6.5						
		16.0	27.5	11.5	9.00	0.61°	4.7			
	d	17.0	25.0	8.0						
		15.0	24.0	9.0	8.50	0.61°	4.9	0.64	4.5	67.6
4. Ringed, leaves removed	*a*	14.0	15.5	1.5						
		12.5	14.0	1.5	1.50	0.455°	2.7			
	d	16.5	18.5	2.0						
		20.0	21.5	1.5	1.75	0.52°	6.4	0.445	2.6	52.2

TABLE 6. *Philadelphus pubescens. Effect of ringing and removal of leaves on osmotic concentration of the stem as compared with the concentration of lower shoots of the same stem*

		Total Gain for Each Pair of Shoots, May 30–June 4	Green Weights	Δ	Green Weight of First Shoots Below	Δ of First Shoots Below
1. Not ringed, leaves present	(*e*)	320	9.5	0.61	10.2	0.615
	(*f*)	350	12.9[3]	0.62	4.4	0.62
2. Ringed, leaves present	(*e*)	310	16.8	0.63	8.2[3]	0.675
	(*f*)	255	8.8	0.63	12.9	—
3. Not ringed, leaves removed	(*e*)	215	10.0	0.585	10.1[3]	0.59
	(*f*)	60	5.3	0.61	6.8[3]	0.57
4. Ringed, leaves removed	(*e*)	30	4.8	0.53	5.3	0.655
	(*f*)	45	6.3	0.59	—	0.66

The cryoscopic data thus far obtained are not sufficiently extensive to warrant a detailed discussion, but they clearly show that the ringing of a defoliated shoot results in a distinct falling off of the osmotic concentration of that shoot. Whether the check in growth is due primarily to a lack of food necessary for energy or for building material, or to lack of water resulting from a deficiency of osmotically active substances, is not clear. From the available data it would seem, however, that the former is of first importance. In a number of instances it was found that the part above a ring would grow slightly, and later would wither completely when the competition for water became more severe. Table 6 indicates, that, as would be expected, the ringing not only decreases the concentration above the ring but also tends to increase that below.

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[3] These are single shoots, not pairs.

These few determinations cannot be fully relied upon for all details, but, assuming that they are approximately correct, it can be readily seen why the part above a ring cannot compete for water with other parts unless it retains its leaves. This therefore would explain the withering of ringed shoots that lack leaves, and shows that the leaves do not directly draw water to the phloem as suggested by Hanstein, but rather that they supply the phloem with osmotically active substances, thus indirectly enabling it to obtain its own water.

It might be stated in this connection that the writer has obtained considerable evidence that one important factor among several possible factors that may be concerned in the matter of polarity and inhibition is associated with a local distribution of foods and of osmotically active substances. The same substance, possibly sugar, may act in both rôles. It has been found, for instance, that the upper part of a rapidly growing shoot may have a concentration that would give a pressure over two atmospheres greater than the concentration in the middle or lower part of the same shoot. The "inhibition" of shoot growth at nodes below the terminal one may be due to a lack of sufficient food and to inability to compete successfully for water rather than to a backward flow of some "inhibitor." In fact, as was shown in a previous paper (Curtis, 1918), it is possible to reverse the polarity by placing the base of a shoot in a strong sucrose solution. The writer has found that other substances are even more efficient than sucrose in thus altering the polarity of a shoot. The check in growth following ringing cannot be due to retardation in removal of an "inhibitor" produced by the leaves, for, if leaves remain, the growth is greater than if they are removed.

The Translocation of Food to Fruits

It has been recognized that at least part of the food carried to growing fruits probably moves through the phloem. Hanstein (1860) found that ringing below fruits, if no leaves were left above the ring, resulted in checking their further development. In order to determine the effect of ringing on the transfer of food to fruits, twelve fairly well matched pairs of young Wealthy apples were selected. The leaves from the stems close to the fruit were removed, and the stem of one of each pair was ringed so that no leaves were above the ring. The greatest length and the greatest diameter of each fruit were taken. The same thing was done with three pairs of Rhode

TABLE 7. *Pyrus malus. Effect of ringing on movement of food to fruit. Leaves removed above ring and to same extent on those not ringed except in No. 3 (o) in which case leaves were left above ring*

		Aug. 6, 1918		Sept. 25, 1918					Relative Gain in Approximate Volume. Check as Unity	Relative Green Weight. Check as Unity	Relative Dry Weight. Check as Unity
		Average of Length and Diameter, Mm.	Approximate Volume, Cc.	Average of Length and Diameter, Mm.	Approximate Volume, Cc.	Gain in Approximate Volume, Cc.	Green Weight, Gm.	Dry Weight, Gm.			
1. Wealthy not ringed	*c*	45	47.66	63	130.77	83.11	113.8	16.74			
	d	43	41.58	45.5	49.26	7.68	63.0	7.82			
	e	40.5	34.74	52.0	83.54	48.80	71.2	10.30			
	f	45.5	49.26	57	96.86	47.60	88.6	11.80			
	i	41	36.05	56.5	94.33	58.28	85.9	11.74			
	j	44	44.55	54.5	84.66	40.11	72.1	10.00			
	Ave.	43.17	42.31	54.75	89.90	47.60	82.43	11.40		1.00	1.00
Wealthy ringed	*b*	41.5	37.38	42.5	40.15	2.77	36.9	4.80			
	d	46.5	52.58	46	50.91	−1.68	47.8	6.66			
	f	41.5	37.38	42	38.75	1.37	38.0	5.05			
	g	41.5	37.38	40.5	34.74	−2.64	33.6	4.98			
	h	37.75	28.14	38.5	29.90	1.76	27.7	3.70			
	i	40.75	35.39	41.5	37.38	1.99	38.0	5.03			
	k	45.5	49.26	46.5	52.58	3.32	47.1	5.48			
	l	41.5	37.38	42	38.75	1.37	39.5	5.06			
	Ave.	42.06	39.36	42.44	40.40	1.03	38.58	5.10	0.02	0.47	0.45
2. Rhode Island Greening not ringed	*m*	48.5	59.67	63	130.77	71.10	121.7	18.43			
	n	48.0	57.84	55.5	89.41	31.57	93.4	13.90			
	o	52.0	83.54	67	157.30	73.76	154.7	21.53			
	Ave.	49.5	67.02	61.8	125.83	58.81	123.3	17.95		1.00	1.00
Rhode Island Greening ringed	*m*	48	57.84	49.5	63.43	5.59	60.5	8.80			
	n	48	57.84	49	61.53	3.69	62.7	8.86			
	o	51	69.38	53	77.86	8.48	77.3	9.48			
	Ave.	49.0	61.69	50.5	67.61	5.92	66.83	9.05	0.10	0.54	0.50
3. Rhode Island Greening ringed with leaves	*o*	52.5	75.68	57	96.86	21.18	92.40	12.61	0.36	0.75	0.71

Island Greenings, and in addition two stems were left without removing the leaves above the ring. The fruit was gathered on September 25 at which time a number had fallen from the trees, which fact accounts for the missing apples. The average of the greatest length and the greatest diameter was taken as the diameter of a sphere and the volume of this sphere was calculated. The data are presented in table 7.

There seems to have been practically no increase in the volume of the apples on the ringed stems. The 2 percent recorded is possibly within the range of probable error. Whether there was a transfer of anything but water to the fruits cannot be definitely determined from the data available, but the fact that both the water content of the fruit from the ringed stem and the volume-dry weight ratio of the same fruit were greater than in the fruit from the stem that was not ringed, as well as greater than in samples taken at the time of ringing, would indicate that no appreciable amount of food had moved to the fruit. The water contents of the fruit from the ringed stem, of that from the unringed stem, and of that taken at the time of ringing were respectively 86.9 percent, 86.2 percent, and 85.0 percent, and the approximate volume-dry weight ratios were respectively 7.92, 7.89, and 7.05.

Effect of Ringing Dormant Twigs in the Spring

Dormant stems of various woody plants were ringed at different distances from the tip to determine whether food stored in the xylem would move longitudinally through the same tissues and to determine from how far

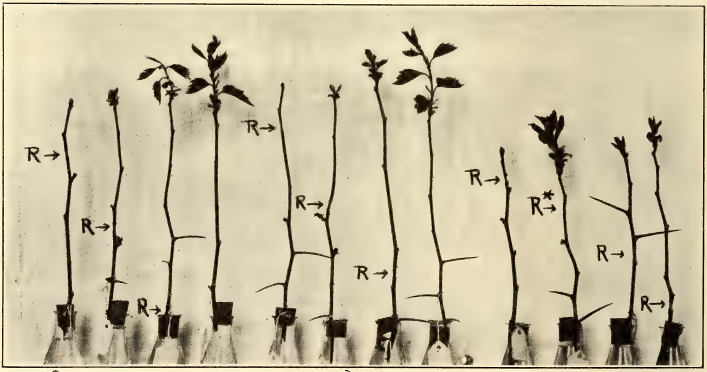

Fig. 2. Crataegus ringed, while dormant, at different distances from the tip. The place of ringing is indicated by *R*. In the stem marked *R** a narrow strip of phloem less than one fourth the circumference was left. Similar results were obtained when only one half of the xylem and one fourth of the phloem were left. (The unringed twig of the group at the right was omitted by the engraver. This twig showed a growth almost identical to that of *R*.*)

back the food supply was drawn. In all cases starch was present in the xylem at the time of ringing. Results obtained when twigs of Crataegus were ringed in this way are shown in table 8.

TABLE 8. *Crataegus sp. Ringed April 8. Measured May 8*

X. Check not ringed.
A. Ringed in 2nd internode from tip.
B. Ringed in 4th internode from tip.
C. Ringed at base of one-year-old wood, usually the 7th or 8th internode.
D. Ringed back on three- or four-year-old wood.

Those marked *z* had half the xylem cut away and three fourths of the phloem. One fourth of the phloem was left.

Lateral buds removed from all.

	X	*A*	*Az*	*B*	*Bz*	*C*	*D*
1.	20	0	—	0	—	16	16
2.	25	14	20	broken	—	16	20
3.	25	2	—	—	—	—	17
4.	25	3	—	8	—	9	—
5.	28	4	—	4	20	—	15
6.	25	6	27	4	—	—	20
7.	35	8	32	8	—	25	30
8.	30	3	—	broken	—	—	—
9.	20	—	—	6	30	20	—
10.	28	6	28	5	—	22	21
11.	27	broken	—	broken	—	—	—
12.	25	—	20	9	—	—	30
13.	30	6	9[4]	10	32	—	—
14.	25	9	10[4]	13	30	—	28
15.	28	7	9[4]	13	—	18	—
16.	30	12	8[4]	16	—	—	25
17.	30	—	30	9	—	10	20
Ave.	26.8	6.1	26.2	8.1	28.0	17.0	22.0

From these results it is apparent that material carried by the phloem is necessary for shoot growth. The check in growth cannot be due to any injury to the xylem, for if half the xylem is cut away and but a quarter of the phloem is left, growth is practically normal. This is shown in the columns *Az* and *Bz* of table 8 and in figure 2. Two possible explanations occur. Either the xylem carries no foods, neither carbohydrates nor nitrogenous material, or the xylem carries certain foods, perhaps carbohydrates, while the phloem carries some other substances, possibly nitrogenous, which may be necessary for growth. If the second alternative were true, the greater the amount of phloem above the ring the greater would be the supply of this substance which may act as a limiting factor. The following data, however (table 9), offer strong evidence that the carbohydrate supply is the limiting factor and that this cannot move upward through the xylem.

[4] These results are not included in the averages, for the wounds were not paraffined and the xylem had dried out checking the growth of the shoot.

TABLE 9. *Crataegus sp. April to May 18, 1919*

X. Check, not ringed.
A. Ringed at the second internode below the terminal bud.
B. Ringed at the fourth internode below the terminal bud.
C. Ringed at the seventh or eighth internode below the terminal bud.
All lateral buds were removed in each case.

No,	*X*		*A*		*B*		*C*	
	Length in Mm.	Number of Leaves	Length in Mm.	Number of Leaves	Length in Mm.	Number of Leaves	Length in Mm.	Number of Leaves
1	65	5	0		0	dried	8.	3
2	70	7	7	(green only	15	2	35	4
3	85	8	0		15	3	30	—
4	45	4	(*a*) 1 (*b*) 0	(green only)	10[6]	1	35	3
5	40	5	0	dried	20	(green only)	35	4
6	35	8	6	(green only)	15	(green only)	10	green
7	70	–	(*a*) 6 (*b*) 30[5]	(green only)	12	(green only)	20	—
8	(*a*) 55 (*b*) 50	5 5	0 60[5]	dried 5	0	dead	30	2
Ave.	57.2	6.2	2.2	0	11.0	0.5	25.4	2.7

Starch tests:

X. Starch fairly abundant in primary xylem, pith, medullary rays, and outer cortex, both near the tip and near the base. In nos. 2, 3, 4, 7, 8*a*, and 8*b*, cambial growth was started and starch had largely disappeared from the outer part of the medullary rays.

A. No trace of starch above rings except in no. 5 which contained traces in the pith. Below rings just as in checks. Nos. 4, 6, and 7 tested for starch in region of ring showed none in upper part, traces in middle, similar to check in lower part.

B. No traces of starch above rings, except in nos. 1 and 8 which had evidently died early. Below rings just as in checks. Nos. 5, 6, and 7 tested for starch in region of ring showed none in upper part, while the lower part appeared about the same as just below the ring.[6]

C. No starch above the rings, except in no. 6 in which the terminal bud had been broken off and adventitious buds were just starting. In three cases there were slight traces in 2–3 pith cells. Below rings starch was fairly abundant as in the check stems.

In each case in which growth had ceased, the starch above the ring had disappeared. If something other than sugar were the limiting factor, one would expect that the starch could not be utilized, yet the shorter the piece above the ring the more rapid was the disappearance of the starch and the earlier the cessation of growth. In a few exceptions with Crataegus and Acer a very short piece above a ring sometimes died before all the starch had disappeared. In the checks, on the other hand, the starch had not all disappeared at the time the data of table 9 were taken. Later, however, as the shoot growth neared completion, starch disappeared from the checks also. It is evident that, when the stem is not ringed, a shoot does not first deplete the starch in its immediate neighborhood, but the starch for some distance is reduced rather uniformly.

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[5] In these twigs a narrow strip of phloem was left covering less than one fourth of the circumference.

[6] In no. 4 a narrow strip of phloem had healed over under the paraffin. Starch was fairly abundant in primary xylem, pith, and medullary rays. None in cortex. Below ring same as in checks.

TABLE 10. *Acer saccharum. Ringed April 7, 1919.*
Ringed in middle of first year's growth

	Growth in Mm. by May 11		Notes
	Ringed Branch	Corresponding Branch Not Ringed	
1...	10	55	*May 11*, nos. 1, 4, and 5 and their corresponding check twigs were cut and tested for starch. *Above ring:* In each case no trace of starch present above the ring; neither directly under the terminal node, in the middle internode, nor in the basal internode. *Below ring:* Starch present in each case in the primary xylem and in the medullary rays in all parts below the rings. *Within the ring:* Starch present as below the ring. *Check:* Starch present in each stem in the primary xylem and in the medullary rays in all parts corresponding to those tested in the ringed stems. *May 16*, all starch had disappeared from check stems as well as from the ringed ones.
2...	20	35	
3...	10	80	
4...	15	90	
5...	12	90	
6...	15	55	
Ave.	13.7	67.5	

Ringed at base of first year's growth.

	Growth in Mm. by May 11		Growth in Mm. by May 24		Notes
	Ringed Branch	Corresponding Branch Not Ringed	Ringed Branch	Corresponding Branch Not Ringed	
1...	45	80	65	205	*May 11*, no. 4 and its corresponding check were cut and tested for starch. *Above ring:* No trace of starch in any part. *Below ring:* Starch present in primary xylem and in medullary rays. *Within ring:* Starch in primary xylem and in medullary rays. *Check:* Starch in primary xylem and in medullary rays in all regions corresponding to those tested in the ringed stems.
2...	45	80	75	180	
3...	50	80	85	210	
4...	45	90	—	—	
5...	35	55	80	240	
Ave.	44.0	77.0	76.3	208.8	

The sugar maple is commonly cited as an example of a tree that transfers its carbohydrates upward through the xylem. The trees were bleeding freely at the time the ringing was done, yet practically no translocation of carbohydrates occurred longitudinally through the xylem.

Very similar results were obtained with the pear, *Pyrus communis*, and the beech, *Fagus grandifolia*. In both, the nearer the ring was to the tip, the less was the growth and the sooner did the starch disappear above the ring. The measurements for the beech are given in table 11 and a photograph of series no. 2 in figure 3.

TABLE 11. *Fagus grandifolia.* *April 7 to May 24*

X. Check twigs not ringed.
B. Ringed in the middle of the one-year-old wood.
C. Ringed at the base of the one-year-old wood.
D. Ringed in the wood three to five years old which in all cases was under one centimeter in diameter.

All having the same number were fairly well matched at the time of ringing.

	X		*B*		*C*		*D*	
	Length in Mm.	Number of Leaves	Length in Mm.	Number of Leaves.	Length in Mm.	Number of Leaves	Length in Mm.	Number of Leaves
1.......	100	5	10	4	20	5	30	5
2.......	130	5	35	4	50	5	30	4
3.......	365	9	50	5	110	7	—	—
4.......	170	6	10	0	17	0	60	6
5.......	240	8	15	0	—	—	100	6
6.......	155	7	15	0	30	0	20	4
7.......	145	6	15	2	30	3	115	5
Ave....	186.4	6.6	21.4	2.1	42.8	3.3	59.1	5.0

One of Hartig's chief arguments that carbohydrates move upward through the xylem was based on the fact that, if a tree was ringed, the starch below the ring disappeared. He assumed that it moved upward, but there is no reason why it could not have been moved radially and used in cambial growth, or moved downward through the phloem to the growing roots. To test this latter possibility, experiments were tried with *Ostrya virginiana*

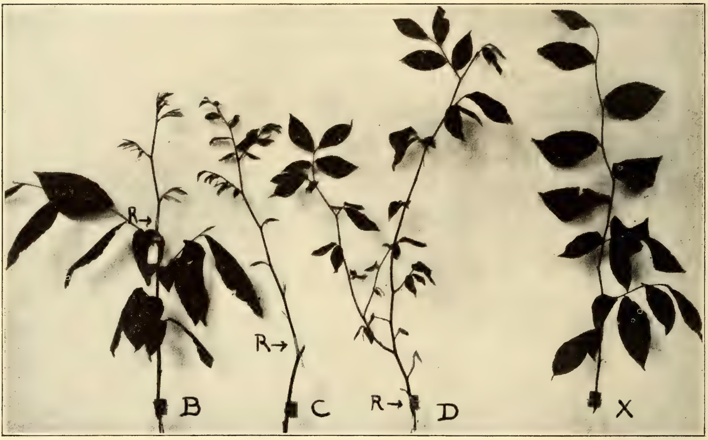

FIG. 3. *Fagus grandiflora* ringed, while dormant, at different distances from the tip. The point of ringing is indicated by *R*.

Koch, *Crataegus* sp., *Acer saccharum* Marsh., and *Fagus grandifolia* Ehrh., in which two rings were made on the same stem some distance apart.

Stems of Ostrya were ringed April 6. By May 6 growth had started and the shoots of the ringed and unringed stems were apparently of the same length, measuring for the most part about 15 to 20 millimeters. The shoots of the ringed stem, however, had used up most of their reserve food, for very little further growth took place while the shoots of the check stem continued to grow. On May 19 two of these stems were cut and tested for starch. For the stem shown in the photograph (fig. 4) the tests were as follows:

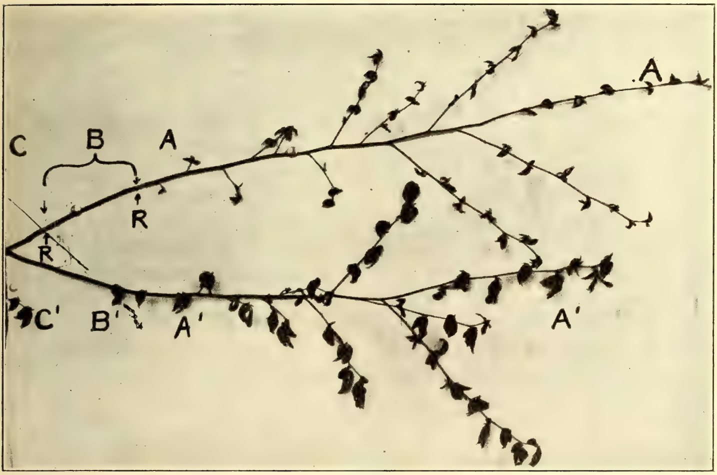

FIG. 4. *Ostrya virginiana* ringed April 6 at *RR*. Cut May 19 and tested for starch with iodine.

Ringed. *A*. Above upper ring. No trace of starch in any part either in the young twigs or in the older stem.
B. Between rings. Starch very abundant in pith, medullary rays, cortex and phloem parenchyma.
C. Below lower ring. Traces of starch in the pith cells. (See page 116 for results as to upper, middle, and lower parts of upper ring.)
Not ringed. *A*. Traces of starch in pith cells.
B. Traces of starch in pith cells.
C. Traces of starch in pith cells.

Above the upper ring: No trace of starch was present in any part, either in the young twigs or in the older stem.

Between the two rings: Starch was very abundant in the pith, medullary rays, and cortex.

Below the lower ring: There were traces of starch in the pith.

Upper part of upper ring: There was no trace of starch.

Middle part of upper ring: There were traces of starch in the pith and in some of the medullary ray cells.

Lower part of upper ring: Starch was abundant in pith and medullary rays, similar to that between rings.

Check: There were traces of starch in the pith cells in the regions corresponding to those listed above.

In the other stem tested at the same time the results were practically the same. There was more starch in the check, however, which showed very distinct traces in the pith and very slight traces in the medullary rays and cortex. In both stems the results were striking. When the sections stained with iodine were held against white paper and observed from across the room, the sections from between rings appeared almost black and the others practically colorless. These results indicate that there is practically no longitudinal transfer of carbohydrates through the xylem.

A similar experiment in double ringing was performed with several species of Crataegus. Stems were ringed April 6 and examined May 17. All showed results similar to those obtained with Ostrya; that is, in all cases starch had disappeared above the upper ring, was abundant between the rings, and was present in moderate amounts below the lower ring and in the check stems. Between the rings, starch was very abundant in the primary xylem and medullary rays; but the results were not as striking as those obtained with Ostrya, for the stem at no time contained as much starch in the pith and therefore did not appear as dark when stained with iodine.

A number of stems of *Acer saccharum* ranging in diameter from 4.5 mm. to 24.3 mm. were double-ringed on April 6 and 7. The distances between the rings ranged from 15 cm. to 107 cm. At the time of ringing most of the stems were bleeding freely. A number of the stems were cut between May 6 and May 19. In all cases starch was completely or almost completely absent above the upper ring, very abundant between rings and nearly absent to fairly abundant below the lower ring, depending on the time of cutting and the position on the tree. The data obtained from one such stem are recorded below and in table 12. This branch showed 15 annual rings at the lower ring; it was 24.5 mm. in diameter at this point, which was situated 16 cm. from the main trunk. At the level of the second ring, which was 107 cm. from the first, the diameter of the branch was 20.2 mm. The check branch was not perfectly matched but was somewhat smaller than the ringed one. The diameter at its base was 22.3 mm.

Above the upper ring: There was no starch in any part except in a few scattering cells of the primary xylem.

Between the two rings: Starch was very abundant in the primary xylem and the medullary rays.

Below the lower ring: Starch was fairly abundant in the primary xylem and in the inner part of the medullary rays. The medullary rays of the outer annual ring were emptied of starch, those of the second contained only traces, those of the third still larger amounts, while in the fourth annual ring the starch was abundant.

Check: At the base starch was fairly abundant in the primary xylem and in the medullary rays of the inner annual rings, disappearing in the third ring and entirely absent in the outer two. Farther out on the stem, at a point corresponding to that of the upper ring on the ringed branch, the diameter was 20.5 mm. At this point starch was mostly absent from the outer nine rings but was fairly abundant in the inner four. Still farther out, where the diameter was 12 mm., starch was present in distinct quantities in the primary xylem only.

The stems were cut in pieces 12 cm. long, which were then peeled to the cambium. These pieces of xylem were centrifuged and the small amount of sap obtained was hydrolyzed with hydrochloric acid and tested for reducing sugar. Samples corresponding to 1 cc. amounts from the stems immediately above the upper ring and between the rings were tested. From above the ring 0.3 mg. of cuprous oxide was obtained on boiling with Fehling's solution, while 2.0 mg. were obtained from between the rings. A second test was lost by accident, but at the time of filtering through the asbestos there was a very distinct precipitate of the cuprous oxide from the sample between the rings and but a very faint trace of the oxide from the other sample. Another accident resulted in the loss of the remainder of the sap, so no further tests could be made.

Samples of the oven-dried wood were sawed into 5 mm. lengths and extracted in 250 cc. of 80 percent alcohol for 72 hours at 37° C. After driving off the alcohol and hydrolyzing with hydrochloric acid by heating to 76°, quantitative tests were made for reducing sugar as shown in table 12.

TABLE 12. *Acer saccharum. Sugars from xylem soluble in 80% alcohol. Hydrolyzed extract made up to 100 cc. 30 cc. samples tested from between rings and] above, 25 cc. samples from the others*

	Dry Weight of Xylem Extracted	Individual Determinations Expressed as Mg. Invert Sugar			Average of Determinations	Mg. Invert Sugar Calculated for 25 Gms. Dry Wood
Above upper ring	23.33	14.15	14.66	14.58	14.46	53.91
Between rings	31.78	59.6	59.85	59.05	59.5	155.84
Below lower ring.....	44.10	55.55	54.38	—	54.965	124.65
Check..............	37.07	40.08	39.10	—	39.59	106.80

From the data shown in table 12 it is evident that the removal of soluble carbohydrates from between two rings does not occur or is very much retarded. The carbohydrates above the upper ring were very much reduced. It is probable that, if a smaller stem had been ringed, the sugar content above the ring would have been very much less; for in such cases shoot growth is quickly checked while in the present instance the shoot growth was apparently no less than in the check. The sugar content below the lower ring was less than that between the rings, showing that from this region some of the carbohydrates had been carried backward to the trunk whence they were carried towards the roots or to branches higher up. The unringed branch showed a sugar content higher than that above the ring and lower than that between upper rings, or below the lower ring, as would be expected.

After the removal of the soluble carbohydrates the residue was treated according to the Sachs method to determine the starch content. The material was boiled for two and one half hours under a reflux condenser in a flask with 10 percent hydrochloric acid of 1.9 specific gravity. The data obtained are recorded in table 13.

TABLE 13. *Acer saccharum. Polysaccharides from xylem hydrolyzed by Sachs method. Extracts made up to 500 cc., 25 cc. of this diluted to 200 cc. and 50 cc. samples analyzed*

	Dry Weight of Xylem	Mg. of Sugar Expressed as Glucose		Average of Determinations	Mg. of Glucose per 25 Gms. Dry Wood
Above upper ring	23.33	49.88	49.02	49.45	4,429
Between rings	31.78	73.00	72.55	72.775	4,580

The data indicate that there are more polysaccharides present in the stem between the rings than above, an equivalent of 151 milligrams of glucose. It is very evident, however, that by this method of extraction much material of the wood that is not starch is hydrolyzed. The iodine test showed only very faint traces of starch above the rings, yet by this method it would seem that this part of the stem contained starch to the extent of about 17.1 percent of the dry weight. It is very probable that pentosanes and possibly other polysaccharides were hydrolyzed.

TABLE 14. *Acer saccharum. Sugars from wood soluble in 95% alcohol. Extract made up to 100 cc., 25 cc. samples taken*

	Determinations Expressed as Mg. of Invert Sugar		Average of Determinations	Total Mg. Soluble Sugar Calculated for 25 Gm. Dry Matter
Above upper ring	12.1	11.8	11.45	45.80
Between rings	31.35	31.35	31.35	125.40

A second set of samples was extracted after a somewhat different manner. Pieces of xylem were sawed more finely into sections about one millimeter thick. Twenty-five gram samples of this dry material, including the sawdust produced in the process, were placed in 250 cc. of 95 percent alcohol and boiled for three hours in a flask with a reflux condenser. The solution was filtered off and the residue was extracted in the same way in fresh alcohol for a second three hours. The filtrate was then freed of alcohol, hydrolyzed with 10 percent hydrochloric acid for three minutes and tested for reducing sugar. The results are recorded in table 14.

Evidently somewhat less sugar was extracted in this way than was extracted in 80 percent alcohol. The ratio of the sugar content between rings to that above, however, is nearly the same in both determinations. These are respectively 2.74 to 1 and 2.89 to 1. The alcohol was removed from the residue by heating under vacuum. Water (300 cc.) was added and the whole was heated to 80°, cooled, and 0.2 gms. taka diastase added. At the time the diastase was added, the filtrate from the wood between rings showed a distinct blue with iodine while the other showed none. After standing 12 hours with taka diastase, 50 cc. samples of each were taken, hydrolyzed 3 minutes with 10 percent HCl and tested for reducing sugar. Calculated as glucose, the sample from between the rings contained 68.05 mg. of sugar and that from above the rings 26.24 mg. The total volumes of solutions were not exactly equal, so that these samples are not strictly comparable. The remainder of the solution was boiled for 3 minutes with 10 percent HCl in contact with the residue. The solution was filtered off, neutralized, and tested for reducing sugar. This is expressed in table 15 as glucose, but it is probable that some maltose remains.

TABLE 15. *Acer saccharum. Sugar obtained from residue by treatment with diastase and acid after removal of sugars soluble in 95% alcohol. Extract made up to 500 cc., 50 cc. samples taken*

	Individual Determinations		Average	Total in 500 cc.	Removed Before Treatment of Residue with Acid	Total for 25 Gms. Dry Wood Expressed as Glucose
Above rings	51.9	51.95	51.925	207.70	26.24	233.94
Between rings	92.25	93.08	92.665	370.66	68.50	439.16

A number of stems of *Fagus grandiflora* were double-ringed and results very similar to those described for Acer were obtained. All the stems tested with iodine showed a large amount of starch between rings and little or no starch above the upper ring. One experiment for which sugar analyses were made will be reported in detail. A young sapling was ringed 10 cm. from the ground and a second ring was made 70 cm. above the first. At this point the diameter of the stem was 30 mm. The rings were about 3 cm. broad. The ringing was done April 7, and the tree, with the check standing within three feet of it, was cut May 27. At this time the shoots had evidently completed their growth, but there was no apparent difference in the growth of the new shoots.

The tests for starch were as follows:

Above upper ring: No starch was present except in a very few scattered pith cells.

Between rings: Starch was very abundant in medullary rays and pith.

Below lower ring: There were distinct traces of starch in the medullary rays and in a few pith cells.

Lower part of upper ring appeared the same as between rings.

Upper part of upper ring: Starch was fairly abundant but distinctly less abundant than in the lower part.

Lower part of lower ring: Starch was fairly abundant in medullary rays and pith but less in the latter than between the rings.

Upper part of lower ring: The starch content was the same as between the rings.

Check: Traces of starch were present in smaller medullary rays and in the pith but less than just below the lower ring. This was true for all parts corresponding to those tested as described above.

The stems were cut into pieces 12 cm. long, from which all tissues outside the cambium were removed. These pieces were centrifuged, and the sap was tested for invert sugar after hydrolyzing with hydrochloric acid. The exact amount of sap remaining after removing 4 cc. samples was not determined as the tubes were rinsed out with water.

TABLE 16. *Fagus grandiflora. Sugar obtained from centrifuged stems*

	Green Weight of Sticks	Mg. Invert Sugar in 4 Cc. Samples	Mg. Invert Sugar in Remaining Sap	Total Invert Sugar Obtained.	Total Mg. Invert Sugar from Centrifuged Sap per 100 Gms. of Xylem
Check	125.0	2.03	1.08	3.11	2.49
Above ring	185.4	1.25	0.72	1.97	1.06
Between rings	219.6	1.67	3.76	5.43	2.47

The amounts of sap obtained were so small that the analyses cannot be fully depended upon, yet there seems to be clear indication that more sugar is present in the sap centrifuged from the xylem between the rings than from that above.

TABLE 17. *Fagus grandiflora. Sugar soluble in 80% alcohol hydrolyzed and expressed as invert sugar*

	Mg. Invert Sugar in 25 Cc. Samples		Average	Total Mg. Invert Sugar in the 50 Gms. Dry Wood
Check	42.34	43.7	43.02	344.16
Above rings	42.30	41.35	41.825	334.60
Between rings	61.08	60.20	60.64	485.12

The wood was sawed into short sections about a millimeter in length. After drying in the oven, lots of fifty grams each of this material, including the sawdust, were extracted in 250 cc. of 80 percent alcohol at 37° for 24 hours, and again in fresh alcohol for 72 hours. After heating to 80° the solution was filtered off, the alcohol was removed by repeated evaporation, and the solution was finally boiled for 3 minutes with 10 percent hydrochloric acid, neutralized, and made up to 200 cc. The amounts of sugar found are shown in table 17.

The residues were shaken up with 250 cc. of water and 0.2 gm. taka diastase. After 12 hours the sawdust still contained abundant starch. The material was then placed in a steam autoclave at 15 pounds pressure for 45 minutes. Two-cubic-centimeter samples diluted with 5 cc. of water and tested with iodine showed deep blue in the sample from between the rings and very faint traces of blue in those from the check and from above the rings. Five tenths of a gram of diastase was added to each and left 18 hours, but on examination starch was still present in the sawdust and in the small sections of wood. The material was then autoclaved a second time at 15 pounds for 10 minutes. On testing the extract as before, only that from between the rings clearly showed the presence of starch.

Without attempting to extract more of the starch, the solutions were filtered off and digested with 0.25 gm. of taka diastase. Each of the extracts was then made up to 500 cc. and 25 cc. samples were tested for sugar. This is expressed in the table as maltose, but it is very probable that much of it was really glucose. Relative values, however, are all that are necessary.

TABLE 18. *Fagus grandiflora. Sugar obtained from residue by heating under pressure and digesting with diastase*

	Single Determinations Expressed as Maltose		Average	Total Sugar Present in 50 Gms. Dry Wood Expressed as Maltose
Check	46.03	40.24	43.14	862.80
Above rings	51.46	52.46	51.965	1039.30
Between rings	89.25	89.78	89.52	1790.40

DISCUSSION

The experiments with ringing accompanied by removal and non-removal of leaves clearly show that some substance necessary for growth passes upward through the phloem and that, if the leaves remain above a ring, they are able to supply some of this substance. This substance may function as a food, supplying building material and energy, or it may function merely as an osmotically active agent, thereby enabling the tissue to compete successfully for water, or, as is more probable, it may function in both rôles. Analyses show that ringing when leaves are removed results in a decrease both of osmotic pressure and of sugar content. Removal of leaves without ringing also decreases the osmotic pressure and the sugar content below those of the normal stem with leaves. The data obtained are not sufficient to enable one to determine with any degree of assurance whether sugar is the only or even the chief substance concerned.

The experiments with ringing dormant stems indicate that the growth above a ring practically ceases when the carbohydrates in this region have disappeared. Even the starch of the xylem itself is not digested and removed upward unless a path through the phloem is open. If that part above the ring is so short that the bud merely elongates but does not open its leaves, growth ceases completely, and later the enlarged bud or partially opened leaves wither and die. If sufficient food was above the ring to enable one or two leaves to open, growth often slowly recommences, probably because of the food made available by the new leaves.

The experiments with double ringing show even more clearly that the xylem does not serve as a tissue for longitudinal translocation of the carbohydrates stored in it, for between rings the content both of starch and of soluble sugars is much higher than either above or below the part thus isolated. It would seem that the removal of carbohydrates from the xylem occurs only radially through the medullary rays and that longitudinal transfer in either direction is through the phloem only. In a ring 1 cm. wide starch was still present when all the starch had disappeared immediately above the ring. Later, however, the upper part had become depleted while the lower part was still full. Diffusion may have accounted largely for the movement through this short distance. Observation on this point has not been made, but it seems probable that much of the food from between rings will be eventually used up in cambial growth.

As was previously stated, Atkins (1916), finding appreciable quantities of sugars in the sap centrifuged from the stems of various woody plants, concluded that these must move upward with the "transpiration stream" and that one of the principal functions of the xylem is to transfer these sugars. The data here recorded, however, show that no appreciable amounts of sugar are transferred vertically through the xylem. In the experiments in which the leaves were removed above a ring, it seems possible that the lack of rapid transpiration might partly account for the failure of the xylem to transfer food; however, in the experiments in which dormant wood was ringed some distance from the tip plenty of leaves were produced above the ring to carry on rapid transpiration, yet this seemed to have no effect in causing the removal of either starch or soluble sugar below the ring, or from between rings in those stems that were double-ringed. In the the maple reported in tables 12–15, and in the beech reported in tables 16–18, there was a very large leaf surface that must have required quantities of water, but even the soluble sugars were not removed from between rings.

It will be necessary to make further tests on centrifuging the xylem before definite conclusions can be safely drawn, but the little that has been done at least suggests that even the sugar in the vessels does not normally move with the water in transpiration. We know that when a tree, such as the maple, is tapped, sugar solution will flow through the vessels. This is not proof, however, that in an uninjured tree there is any flow of solution through the vessels. If this were true, one would expect a depletion of carbohydrates below a ring in the maple, especially when it is ringed during the season of sap flow and when the ring is only one to two centimeters broad.

A possible frequent interposition of living cells across conducting tubes may readily prevent a flow of solution, and the water may normally move largely by diffusion. When a tissue is cut, however, as in pruning or in tapping, there is probably an actual flow of solution through the opened vessels which might also result in a depletion of storage materials from adjacent tissues. The movement of water through uninjured xylem may occur chiefly by diffusion as in the case of the entrance of water from the soil into a root. In the latter case there is certainly no flow of solution, as the amount of water taken up has no relation to the amount of solutes absorbed. Data reported by Kiesselbach (1916), who grew plants in soil, indicate that increased transpiration does not increase ash absorption. Experiments by the writer in collaboration with Dr. E. Artschwager and Dr. N. B. Mendiola, that are nearly ready for publication, show that doubling the transpiration from plants growing with their roots in nutrient solutions has no tendency to increase salt absorption.

It may even be that at least some of the mineral nutrients move primarily through the phloem. It is true that, if a stem is placed in a solution of dye, the dye will rise rapidly through the xylem, but whether it will do so in a normally rooted plant has not been conclusively proven. The writer has found, however, that lithium chloride applied to a rooted plant will move through the xylem past a ring. It is possible, however, that it was not carried in the "transpiration stream" but rather that it moved past the ring by diffusion. Lithium salts will diffuse much more rapidly than sugars, and cell membranes seem to be very permeable to them. The lithium was found in the phloem and cortex above the ring as well as in the xylem.

A number of papers have been published in which it has been shown that certain dyes or salts appear to travel in the "transpiration stream" through the xylem when the roots of the plant are placed in the solutions. But in many cases the dyes or salts used may have had toxic effects as a result of which the cells that might normally retard their movement may have been killed or their permeability may have been increased. Furthermore, it has been found in a number of instances that the solutes appeared to travel at about the same rate in the phloem region as in the xylem. Bokorny (1890) found that, when roots were placed in solutions of iron sulphate or of various dyes and the leaves were exposed to very drying conditions, the solutes were found in the phloem region at about the same height as in the xylem region. When on the other hand a cut stem was placed in the solution, the movement through the xylem was distinctly the more rapid. Because he always found the solutes not in the lumina but in the walls of the thicker-walled elements, such as the vessels, sclerenchyma, and collenchyma cells, Bokorny came to the conclusion, which has since been clearly disproved, that water moves through the walls by imbibition. Though his conclusions are of little value, his data at least suggest that solutes are not necessarily carried by the "transpiration stream."

It will be necessary to conduct experiments using rooted plants and various non-toxic salt solutions before any very definite conclusions can be drawn as to the region of transfer of salts in plants.

Summary

Defoliated stems from which a ring of tissue extending to the cambium is removed cease to continue growth.

This cessation is due to the inability of the xylem to carry the necessary food.

This food is needed not only to supply energy and building material but also to increase the osmotic concentration of the tissues, thereby enabling them to absorb water.

This food consists, at least partially, of carbohydrates.

If the stem above a ring is not defoliated, the leaves are able to supply sufficient of this food to allow for considerable growth.

When dormant stems are ringed, the growth above the rings ceases soon after the starch supply is depleted, and the greater the supply of starch above a ring the longer will growth continue.

The carbohydrates stored in the xylem below the ring can not be removed through the xylem but must be transferred radially to the phloem, where they may be carried downward if there is no second ring below.

The carbohydrates of the xylem between two rings remain there at least for some time after those above the upper ring and those below the lower ring have been mostly removed.

Although large amounts of carbohydrates are stored in xylem tissues, there is no appreciable longitudinal transfer of sugars through these tissues.

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EMBRYO DEVELOPMENT AND POLYEMBRYONY IN RELATION TO THE PHYLOGENY OF CONIFERS[1]

JOHN T. BUCHHOLZ

A consideration of embryogeny has played an important part in nearly all discussions of phylogeny, but the embryo development of conifers has offered so many variations and apparent anomalies that many students of Gymnosperms have been in doubt as to whether the embryogeny should be considered very seriously in connection with a study of the morphology of this group. So far as I know, the question of polyembryony has not generally entered into these comparisons, but has been looked upon as an extremely variable feature and one of little morphological importance. The present discussion is an attempt to take into account both the embryo development and polyembryony in making comparisons between the embryogenies of conifers, and aims to point out how these features of embryo development may prove to be very valuable criteria in arriving at the true phylogeny of the Coniferales.

Since my new interpretation of the embryogeny of conifers involves an accurate knowledge of the condition known as polyembryony, it will be necessary to consider briefly the events of proembryonic development that lead up to and accompany this condition. We will use Pinus as our first illustration, because its better known details of development serve as an excellent standard of comparison for the other conifers. I expect to show, further, that the embryogeny of Pinus occupies a very primitive position in relation to all conifers whose embryogeny has thus far been described.

PINUS

In the proembryo stage of Pinus it is well known that walls appear following the mitosis between the four- and the eight-nucleate stages, but it should perhaps be emphasized that after these first walls are formed (fig. 2), the cells of the lowest tier (*p*) are the initial cells which give rise to the four distinct embryos that completely separate from each other. Another free nuclear division occurs in the tier of incompletely walled cells above

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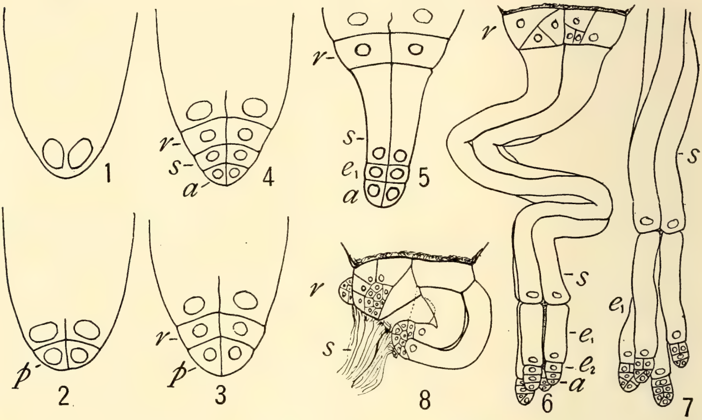
this, and hence the tier of cells *r* (fig. 3), the rosette tier, is also organized directly after a free nuclear division in the proembryo. These rosette cells are now known to be embryo initials (2).

In this stage, shown in figure 3, with the upper aborting tier of four free nuclei and with eight-walled cells beneath them arranged in two tiers, we have the initial cells of all the embryos that are regularly produced from a single fertilized egg in the pine. Of course, some of these initials, especially those of the rosette group, may abort in this stage or in any subsequent stage of development.



Figs. 1–8. Embryogeny of Pinus. Figs. 1–4. Sectional views of proembryonic stages. Fig. 5. Embryos when suspensors begin elongating. Fig. 6. Separation of four embryos coming from *p*. Embryos arising from rosette (*r*) are shown in figure 8. *a*, apical cell; *s*, suspensor; e_1, e_2, embryonal tubes that add to suspensor; *p*, tier of initial cells of primary embryos; *r*, rosette tier.

The embryos that arise from each of these initial cells begin their development by true apical cell growth; first the apical cell has only one cutting face, and later, when the embryos have separated, it has three cutting faces. The apical cell vanishes, usually before an embryo of more than 500 cells is formed.

Following the organization of these eight walled cells, the embryo initials of the tier *p* undergo simultaneous division in which the tier *s* is formed (fig. 4). These first segments (*s*) of their respective apical cells elongate to form suspensor cells (figs. 5, 6), while the apical cells (*a*) form additional segments, e_1, e_2, etc., that elongate and add to the suspensor.

By this time the four vertical rows of cells, representing as many embryos, have separated, and the rosette cells (*r*) begin to proliferate to form the rosette embryos. The four rosette embryos usually do not actually separate (fig. 8), but are nevertheless entirely independent of each other in their further development, while the four primary embryos completely separate from each other, eight embryos being the normal product of each egg in Pinus.[2]

Cleavage Polyembryony

This separation of the zygote into a number of smaller units which undergo competition with each other is called cleavage polyembryony, to distinguish it from the simple polyembryony that may result from the fertilization of several eggs. The free nuclear divisions occurring during the proembryo stage in Pinus are followed by the equal cleavages which organize the initial cells of each of the embryos that separate. It is well known that only one of these many embryos, produced either by cleavage polyembryony or by simple polyembryony survives to the maturity of the seed. This is surely a "survival of the fittest" if vigor in the embryo is any sort of measure of future fitness.

This cleavage polyembryony with very definite proembryonic organization, which is present in Pinus, is apparently a primitive character in the development of this group of seed plants. This character tends to be modified or eliminated, reverting to the condition of simple polyembryony, as we advance along several phylogenetic lines, and is lost by the time the level of the Angiosperms is reached. If a splitting of the embryo is found among Angiosperms, which is rare, it is a cenogenetic character, a condition which has not necessarily been carried over from Gymnosperms.

It is well known that the term polyembryony when applied to Angiosperms has no such definite meaning as when applied to Gymnosperms, for among conifers the term has been used to designate the plurality of embryos which arise from the cleavage of one egg (cleavage polyembryony), or from the fertilization of several eggs (simple polyembryony), but not otherwise. Among Angiosperms, as summarized by Coulter and Chamberlain (12), the embryos are known to arise from a plurality of eggs in only two species (widely separated in phylogeny), occasionally by a process of budding from the suspensor, and in only one species by the splitting of an embryo derived from the single egg. Furthermore, the extra embryos are derived from synergids in about twelve species, from nucellar or other tissue outside of the embryo sac in eleven or more species, from antipodal cells and even from endosperm in others, while false polyembryony may occur through the fusion of ovules, etc. Clearly this Angiosperm polyembryony has little in common with the phenomenon when found in Gymnosperms.

In all Gymnosperms, polyembryony results in a selection of a single embryo from a larger number, long before the seed is matured, while in Angiosperms it is a common thing to find that several embryos survive to the maturity of the seed. The suspensor of the Gymnosperm embryo is an organ of competition, the structure upon whose merit the selection of the surviving embryo depends, while in Angiosperms it is usually a chain of comparatively unelongated cells which carries the embryo into the central portion of a very soft endosperm. Ginkgo, which has a relatively short suspensor, seems to be about the only Gymnosperm which occasionally matures more than one of its several embryos, and even here Cook (11) found this occurring in only two percent of the seeds. It is much more infrequent than this in Pinus, and probably in all other Gymnosperms. It is clear that the erratic character of polyembryony in Angiosperms should not influence our judgment in deciding on the phylogenetic value of this feature in Gymnosperms.

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[2] A more detailed review and discussion of the proembryonic development of Abietineae is given in another paper by the author (4).

Simple polyembryony is found among cycads, since these have constantly several archegonia; but none of them are known to possess cleavage polyembryony with its accompanying phenomena, so that polyembryony is a feature too uniform to be of value in showing the affinities of the genera within the Cycadales. However, the ordinary anatomical characters of the proembryo have been used very effectively for this purpose by Chamberlain (6).

Among conifers, much more precise comparisons of embryonic development are possible when we once understand these greater variations of the proembryo and of the early embryo brought about by cleavage polyembryony; stages of development which one would otherwise expect to find rather conservative and uniform. The peculiar splitting of the embryo, which was introduced somewhere in the ancestry of Pinus, has persisted for some time in the evolution of the conifers, and was suppressed or eliminated by a number of distinct methods. The rosette embryos, rosette cells that abort, and many other early embryo features are only results of cleavage polyembryony. Together with the apical cell (doubtless a Pteridophyte character which has persisted), these features give us a splendid array of consistent characters to serve as an index to the natural classification of the groups.

ABIETINEAE

In a very recent paper on "Polyembryony among Abietineae" (4), I have made a number of comparisons of the embryos of this group, which are summarized with greater accuracy in the diagram of figure 9. While the proembryos of all Abietineae (with the possible exception of Pseudotsuga) appear to be identical (4), the condition of cleavage polyembryony (*Cl.p*) is restricted to Pinus, Cedrus, and Tusga. *Abies balsamea* displays an occasional embryo with cleavage polyembryony, but normally it has only simple polyembryony and is similar to Larix, Picea, and Pseudotsuga in this respect.

It will be seen that the apical cell (*A*) has the same range of distribution within this group. Fusion of embryos seems to be the method by which both of these characters disappeared from the ontogeny of the higher Abietineae.

Rosette embryos (*R-em*) usually develop in Pinus, are somewhat less developed in Cedrus, while Abies has rosette embryos only rarely, about as often as it has cleavage polyembryony. However, these two occasional characters of Abies are not necessarily associated with each other in their occurrence.

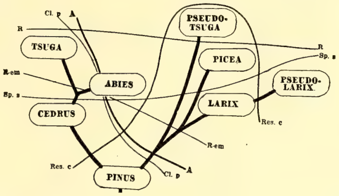


FIG. 9. Diagram to show the affinities of the genera of Abietineae, based on embryogeny. *A*, probable range of apical cell; *Cl.p*, range of cleavage polyembryony; *R-em*, range of rosette cells which may give rise to rosette embryos; *R*, range of abortive rosette cells. Two anatomical characters are included: *Res.c*, the range of resin canals in secondary wood, defining the Pinae of Jeffrey (19, 20); *Sp.s*, range of spur shoot, used by Engler and Gilg (16) and others in subdividing the Abietineae.

In Picea, Larix, and *Abies balsamea* (normally) the rosette embryos are represented only by their initial cells (*R*). Tsuga also has only these aborting rosette cells, though its primary embryos separate as in Pinus. These abortive rosette cells are the last rudimentary structures that remain to indicate the origin of these embryos with simple polyembryony from the type of cleavage polyembryony found in Pinus.

One of the most formidable obstacles to the opposite interpretation, namely, that the embryo of the Picea or Larix type has given rise to the pine type with its cleavage polyembryony, comes from the study of these rosette cells. It is impossible for these abortive structures as represented in Picea or Larix to have given rise to the active rosette embryos of Pinus and Cedrus. On the other hand, the view that rosette embryos and the abortive rosette cells are steps in the elimination of cleavage polyembryony offers a very satisfactory hypothesis that is consistent with the facts and that very definitely points out the direction of this polyembryonous evolution.

In Pseudolarix the rosette cells are present (29), but nothing has been described or figured to indicate whether cleavage polyembryony or rosette embryos develop at a later stage. The exact position of Pseudolarix is still doubtful, but from what is known of its embryo, and considering that it resembles Larix in its spur shoot, it is safest to place it in the diagram near Larix.

In Pseudotsuga, the uppermost walled cells of the proembryo elongate to form the suspensor, and therefore no aborting rosette cells are found.

It is interesting to note how these embryo characters relate themselves to the results of the anatomists. The distribution of the resin canal characters described by Jeffrey (19, 20) are included in the diagram (*Res.c*), cutting Pinus off from Cedrus, although these two genera are very similar on the basis of embryongeny and also in the spur shoot characters used by Engler and Gilg (16) and others as a basis for subdividing the Abietineae. On the other hand, the distribution of the apical cell, cleavage polyembryony, and rosette embryos cuts Pinus off from the rest of the Pinae of Jeffrey. The intermediate position of Cedrus between Pinus and Abies was pointed out by Jeffrey (19) and by Chrysler (7) on the basis of medullary rays. Pinus has such an array of primitive features, both embryological and anatomical, that there can no longer be much doubt of its primitive position in any natural phylogeny of Abietineae.

OTHER CONIFERALES.

In the light of the foregoing, let us now consider the affinities of the other Coniferales as revealed by their embryo development, where this is sufficiently known. The interpretations which I shall give, while not generally those given by the investigators to whom the particular work is credited, do no violence to the facts as they have been described, and for any errors due to these interpretations I assume full responsibility.

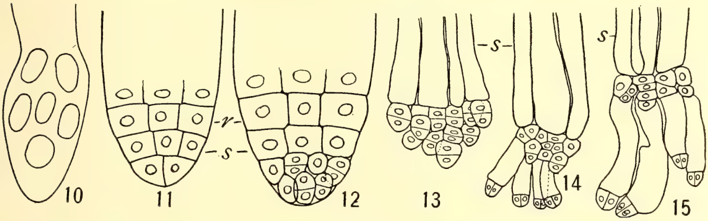
I will first present the line of evolution between Pinus and Araucaria, based on embryogeny. There are not many known types that fit in between these two very different methods of embryo development, but we have steps enough to give us a definite clue to the possible lines of advance, and to suggest the kind of embryogeny from which that of the araucarians was derived.

The number of free nuclear divisions is increased and a larger number of embryo initials is formed, resulting for a time in greater cleavage polyembryony. Advancing a little further, we find that only a small portion of these potential embryo initials function. Cleavage polyembryony is then eliminated by the modification of the terminal portion, which organizes a cap and prevents the embryos from splitting apart. The apical cell also persists for a time in this line of evolution, and it appears that the formation of this cap over the advancing end of the embryo is the thing which does away with the apical cell stage. Here again we have probably had the apical cell stage and cleavage polyembryony eliminated by the same device.

Even if we take the position that Pinus did not directly give rise to araucarians, it is apparent that they had a common origin and were derived from a condition of cleavage polyembryony. Pinus has remained in this condition with very little modification, while the araucarian embryogeny has become specialized, and has completely eliminated cleavage polyembryony. A few stages in this araucarian line of evolution may be illustrated by Sciadopitys and the podocarps.

SCIADOPITYS

The embryogeny of Sciadopitys (figs. 10–15) has been partially described by Lawson (27) and Arnoldi (1), and represents a step in the direction of Araucaria. There are at least eight free nuclei before walls form (see fig. 10), and Lawson states that "eventually the proembryo consists of three tiers of cells and one tier of free nuclei," from which I have supplied fig. 11. However, the organization of embryo initials continues in the terminal tier until a group of about 16 cells is formed (fig. 12). (This stage is also supplied from Lawson's description and from a study of the next stage (fig. 13) by Arnoldi.) Finally the suspensor tier elongates and thrusts the terminal group of cells into the gametophyte, a stage shown in figure 13. That the cells of this terminal group are really embryo initials, mostly advanced to the two-celled stage, is borne out by the next two figures.



FIGS. 10–15. Stages in embryogeny of Sciadopitys. Figure 10 after Lawson (27) figures 11 and 12 supplied from description by Lawson; figures 13–15 after Arnoldi (1).

It will be noted that in Sciadopitys a large number of embryo initials are produced, and the functional ones are organized in this instance *after* the first walls have formed in the proembryo; that is, the lowest functional group is not the first group of embryo initials to organize, as in Pinus, but the last. I would look upon a late organization of the functional embryo initials from previously walled cells as a more advanced condition, for this occurs quite generally in the more recent conifers.

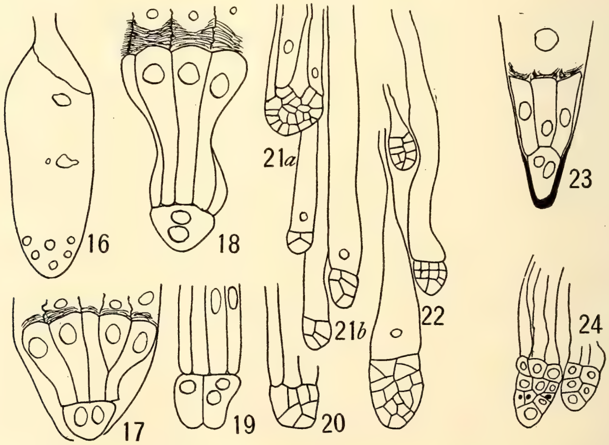
Rosette embryos undoubtedly exist in Sciadopitys, as Lawson accounts for walled cells above the suspensor, and Arnoldi definitely described a group of proliferating cells occupying this position which he noted as a possible vestigial protocorm, comparable to the protocorm of lycopods. He states: "Ich kann gewiss nicht bestimmt entscheiden, ob es ein morphologisches Gebilde ist, oder eine Anpassung zur noch weiteren Polyembryonie." Unfortunately Arnoldi gave us no figures of this "protocorm," but it is probably safe to infer that he saw a group of rosette embryos, and that the entire embryogeny of Sciadopitys represents a modification in the direction of greater cleavage polyembryony.

PODOCARPINEAE

The embryo development of the podocarps, according to the meager descriptions available, represents a condition much further removed from Pinus, one which is to be looked upon as modified from a form intermediate between Sciadopitys and the araucarians. In many respects, these podocarp embryos illustrate some of the general tendencies of embryo modification which brought about the highly specialized araucarian type, and are therefore considered here.

The proembryo of Podocarpus (fig. 17) consists of two very unequal tiers of cells, and some free nuclei in an open tier above. Figure 18 shows an early proembryo with suspensors elongating, and with its binucleate terminal cell undergoing further division in figure 19 before the embryo initials, which may separate, are formed. From the fact that these suspensor cells in this case were organized from free nuclei, one could infer that these are suspensor-forming embryo initials. The rosette cells of Pinus, which certainly are embryo initials, were sometimes found to elongate as suspensors (2), and we might expect to find some groups of conifers in which such unfavorably placed embryo initials are normally modified to form suspensors.

While *Podocarpus coriaceus* (figs. 16–22), which was investigated by Coker (8), produced walls only after sixteen or more free nuclei were formed,



FIGS. 16–24. Stages in embryogeny of *Podocarpus coriaceus*, after Coker (8). FIG. 23. *Podocarpus nivalis*, showing binucleate terminal cell enclosed by a thick cellulose cap. FIG. 24. Embryos of *P. ferrugineus*. Apical cell stage appears to exist in figures 20 and 21*b*–24. Figures 23 and 24 after photomicrographs by Sinnott (39).

Sinnott (39) described *P. totara* and *P. nivale* as producing walls at an earlier stage, which fact places the Podocarpineae a little closer to the Abietineae.

Coker states that either the embryo may split up or its parts may unite in producing the embryo, but it is highly probable that cleavage polyembryony is the normal condition in the species which he investigated. All his figures of embryos that presumably were produced by simple polyembryony appear very much like those which one would expect to see in the older separated embryos which have a secondary suspensor, and we know that all conifers produce such secondary additions to the suspensor by the elongation of cells to form embryonal tubes. From the interlocking articulation of the embryo of figure 21*a* with its suspensor, it is evident that these are embryonal tubes and not the primary suspensor, as inferred by Coker; but the largest embryo of this group is not the terminal one in this particular instance.

It is also possible that the apical cell stage is found in these separating embryos of Podocarpus, as may be seen by a careful study of figures 21–24. No rosette cells are found in the above described species (I refer to walled rosette cells, not the free nuclei that abort above).

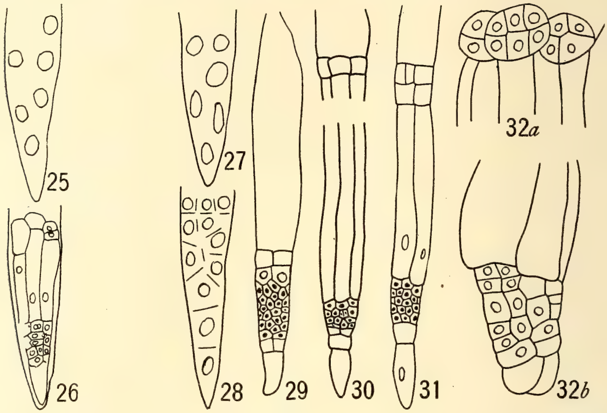
In *Podocarpus nivalis*, Sinnott described and figured a thick cellulose cap "protecting" the binucleate terminal cell, as shown in figure 23, which is pushed far into the gametophyte before it divides further, and "instances of budding or of single suspensors giving rise to embryos were only rarely observed." Does it not appear that this thick cellulose cap would tend to prevent a splitting of the embryo and so to cause the initial cells of the lower tier to combine and produce a single embryo?

I have occasionally observed a cap of wall thickening which appeared to hold together the terminal cells in the embryo of Pseudotsuga,[3] but this structure is not found in the slightly later stages. It is evident that cleavage polyembryony occurs only with considerable difficulty where such a thick cellulose cap protects the terminal group of cells. I am inclined to look upon these caps as mechanical devices which prevent cleavage polyembryony.

Another type of embryo development, probably a higher specialization of that above described, is also found in the Podocarpineae. I refer for illustration to the embryo of *Podocarpus spicatus* (figs. 25–26), one of the Stachyocarpus group (39), in which the terminal cells organize into a cap while the cells above the cap give rise to a single embryo and its suspensor of embryonal tubes. In this case we have no cleavage polyembryony, and it appears that the function of this cap, which is soon sloughed off, may be to prevent this splitting of the embryo, a danger present only in the first stages. However, it would probably not prevent cleavage polyembryony as successfully as the more elaborate caps found in Araucaria and Agathis.

[3] Unpublished work.

The Cephalotaxus embryo shown here in figures 28–32 doubtless has been modified from something like this *Podocarpus spicatus* type of embryo. It has, however, a feature which would relate it more directly to Pinus namely, a conspicuous group of rosette embryos (fig. 32*a*) above the suspensor. The figures here shown are taken from Strasburger (41), who delineated this feature without comment. A similar condition of the rosette may yet be found in the podocarps, for it seems to be suggested by figure 26.



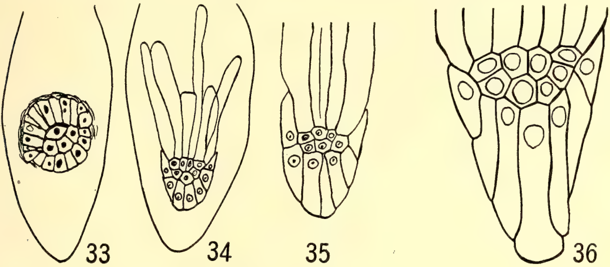
FIGS. 25–26. Stages in embryogeny of *Podocarpus spicatus*, showing terminal cap cell, suspensor cells beginning to elongate, and the group of embryo-forming cells between. After photomicrographs by Sinnott (39). FIGS. 27–32. Stages in embryogeny of Cephalotaxus, showing terminal group of cap cells, embryo-forming cells, suspensor, and what is probably a group of rosette cells in 32*a*. FIG. 27. *C. drupacea*, after Lawson (25). FIGS. 28–32. *C. Fortunei;* figure 28 after Coker (10), and figures 29–32 after Strasburger (41).

The group of cap cells in this case is probably also a device to prevent cleavage of polyembryony or apical cell growth, for this cap is also sloughed off (fig. 32*b*) soon after the proembryo stage, the stage in which cleavage polyembryony is found, if at all.

ARAUCARINEAE

While I would not derive the araucarian type of embryo with its elaborate cap (figs. 33–36) from these podocarp embryos, it is probable that these two groups had a common origin, that the caps of the embryos of both were derived in response to similar conditions, and that these peculiar structures in both instances serve to prevent cleavage polyembryony. The increase in the number of free nuclear divisions in the proembryos of both these groups may be due to a past history of greater cleavage polyembryony, which might well have been overcome by a mechanical device.

In the Podocarpineae and Cephalotaxus, the functional initials were reduced by the abortion of a large portion of the free nuclei in the proembryo, the walled cells below these became suspensor-like, and cleavage polyembryony was eliminated by the organization of caps of various kinds; while in the Araucarineae all the free nuclei are utilized and become organized into an embryo whose terminal portion has a multicellular cap, as shown in figures 33–36.



FIGS. 33–35. Stages in embryogeny of *Agathis australis*. FIG. 33. Proembryo after wall formation, × 200. FIG. 34. After cap is formed and suspensor begins to elongate, but before archegonium is filled, × 260. FIG. 35. Section through tip of older embryo showing the small cells below the suspensor from which the embryo is derived, × 460. After photomicrographs by Eames (15). FIG. 36. Embryo of *Araucaria brasiliensis*, drawn to half the scale of figure 35, × 230. After photomicrograph by Burlingame (5).

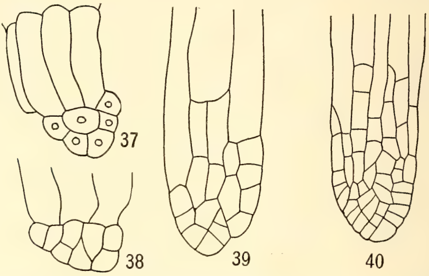
These embryos of Agathis and Araucaria were very satisfactorily described by Eames (15) and Burlingame (5), and neither of these investigators found cleavage polyembryony. In both these species the free nuclear divisions of the proembryo are essentially the same. The proembryo is confined to a samll portion of the egg cytoplasm, and the embryo has begun to elongate considerably before the archegonium is filled by its tissue. The entire embryogeny of the Araucarineae bespeaks a high degree of specialization.

That the cap is not particularly useful in protecting the embryo in its penetration of the gametophytic tissue was brought out by Burlingame (5), and the possibility that it is a special secretive organ is just as remote; but, since other conifers appear to have developed mechanical devices to prevent cleavage polyembryony, an explanation of this nature is possible.

An explanation which would derive the Abietineae from the Aracuarineae on the basis of embryogeny is beset with great difficulty, if not impossible; but it is not so difficult to show how the araucarian embryo has developed from a form very similar to that of Pinus as a specialization along a particular line. At the same time this latter theory offers a satisfactory explanation for the nature of the cap.

TAXADS

The taxad line may have been derived from some of the podocarps, or more probably along with these from a condition nearer to that of Pinus. In Taxus (18) there are at least 16 free nuclei before walls form, and the terminal tier of the proembryo may contain several cells, probably all of them potential embryo initials. Apparently one of these several cells, doubtless the one most favorably situated, begins to cut off apical cell segments and gains the ascendency over the others (40), which in turn contribute by their activity to the suspensor. Figures 39–40 by Strasburger (40) show that the apical cell persists for some time, and this furnishes an instance of the elimination of cleavage polyembryony with the retention of the apical cell.



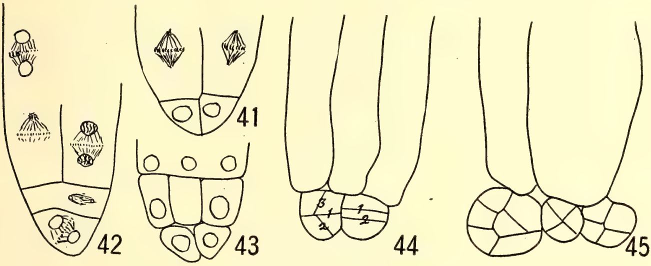
FIGS. 37–40. Stages in embryogeny of *Taxus baccata*, × 125. FIG. 37. Proembryo at beginning of suspensor elongation, after Hofmeister (17). FIG. 38. Slightly later stage. FIGS. 39 and 40. Later stages showing apical cell in early embryo. Figures 38–40, after Strasburger (40).

According to Jäger (18), some of the upper suspensor cells of Taxus may occasionally break away and appear to give rise to small secondary embryos, but practically always the proembryonic cells all combine into a single embryo. Thus, *Taxus baccata* appears to have practically overcome cleavage polyembryony, but still shows some vestigial evidence of an origin from this condition. In commenting on this Jäger (page 182) makes the significant statement that it appears to him that occasionally all the cells of the early proembryo show a capacity for giving rise to embryos, and in this statement he seems to have approached very close to the present explanation.

In Torreya, the proembryonic tissue fills the entire egg, while in Taxus it is confined to the lower portion of the egg. Torreya has only four free nuclei before walls form, and, though no cleavage polyembryony occurs, the vestigial evidence of it in the form of occasional secondary embryos has been described (14). The differences between Taxus and Torreya may be looked upon as due to higher specialization of the latter, and it is therefore questionable if the apical cell stage exists in Torreya.

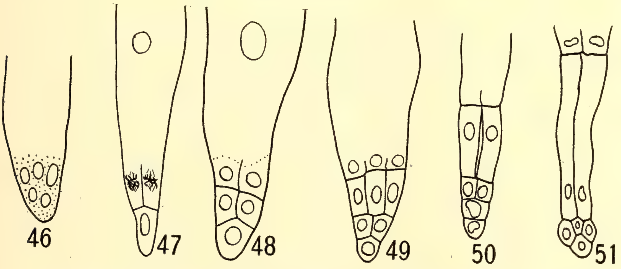
Taxodineae

Among Taxodineae, the excessive cleavage polyembryony described for Sciadopitys becomes reduced, as illustrated by Taxodium (9) (figs. 41–45). Figure 43 doubtless represents two unequal tiers of embryo initials in addition to the upper aborting nuclei. The lowest tier forms separate embryos (fig. 44), while the next tier of initials above it forms suspensor cells. Cleavage polyembryony is apparent. It is probably the normal condition, and we sometimes have the organization of embryo initials after the first appearance of walls, as shown in figure 42. Coker shows the order in which the walls appear in the embryos of figure 44, which is a good start for an apical cell stage. In general the embryo of Taxodium shows great similarity to that of Pinus, except for the unequal organization of the tiers in the proembryo (see figs. 41 and 43) and the absence of the rosette cells.



Figs. 41–45. Stages in embryogeny of *Taxodium distichum*, × 200. After Coker (9).

Cunninghamia (28) may be a step higher, for here it is highly probable (judging from the few stages described by Miyake (figs. 46–51)) that only the lowest embryo initial gives rise to the embryo. This may have been the method of overcoming cleavage polyembryony in this line of evolution,



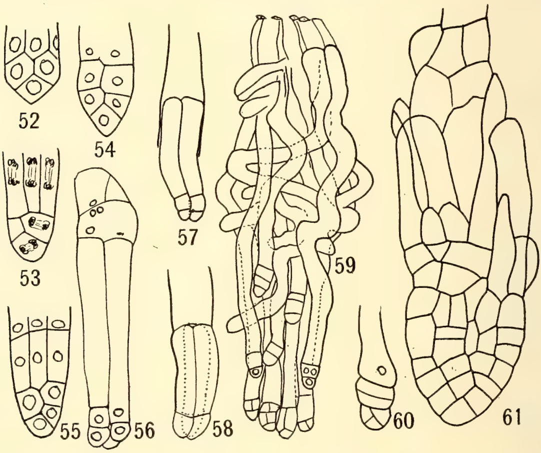
Figs. 46–51. Stages in embryogeny of *Cunninghamia sinensis*, × 110. After Miyake (28).

the functioning of only the lowest embryo initial, the others giving rise to the suspensor. Whether or not an apical cell organizes in Cunninghamia remains to be discovered.

Cryptomeria (24) belongs somewhere in this group on the basis of embryogeny, but the account and figures given by Lawson are not complete enough to warrant any definite conclusions.

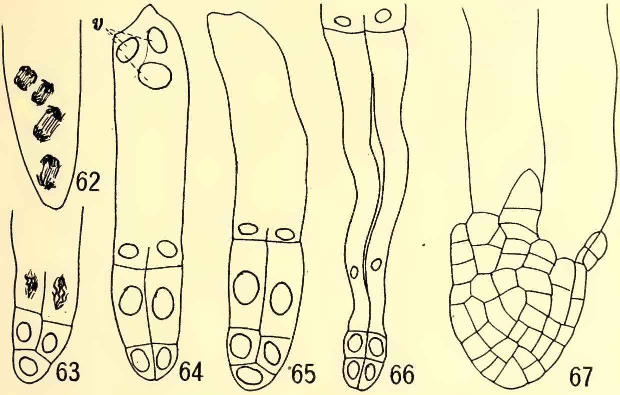
Cupressineae

In the Cupressineae, the proembryo also resembles that of Pinus except for the unequal tier organization. In Juniperus (figs. 52–61), which represents the condition nearest to Pinus, the proembryo seems to organize with the usual embryo initials in several tiers and, above, some inactive nuclei that abort (17, 30, 31, 40). The walled cells below these aborting nuclei function as suspensors, while the lowest group of embryo initials becomes organized into embryos that separate. Figures 56–59 show that cleavage polyembryony is found, and figures 59–61 that the apical cell stage is probably the normal condition.



Figs. 52–61. Stages in embryogeny of Juniperus. Figs. 53 and 55. *Juniperus communis* var. *depressa*, × 150, after Nichols (30). Figs. 52, 54 and 56. *J. communis*, after Noren (31). Fig. 58. Same, after Hofmeister (17). Figs. 57, 59–61. *J. virginiana*, after Strasburger (40). Figure 59 shows the many embryos arising from an archegonial complex, multiplied further by cleavage polyembryony, × 75. Figures 56–58 indicate that an uneven tier arrangement (shown in figures 52 and 54) is no hindrance to cleavage embryony. Figure 53 shows that embryo initials may organize *after* the first walls form, and figures 60 and 61 (× 185) show the apical cell.

Thuja (figs. 62–67) is a step higher, for here cleavage polyembryony does not occur normally. I find from my own studies[4] of this species that it occurs occasionally, probably coming from such embryos as shown in figures 64 and 66 by Land (21). In most cases, one of the embryo initials, such as the lowest of figure 65, has a more favorable position and gives rise to the embryos, the others only elongating to form the suspensors.



FIGS. 62–67. Stages in embryogeny of *Thuja occidentalis.* Figures 62–66, after Land (21), × 300. FIG. 87. Later stage showing apical cell of embryo, × 225, after Strasburger (40).

Whether only the terminal embryo initial functions or several of them, an apical cell stage is always found; a very conspicuous feature in Thuja (fig. 67). From my own studies[4] of *Thuja occidentalis,* I can also state that rosette embryos are found in a significant number of cases, but they are not found in *Thuja orientalis.*

Tetraclinis probably represents a more advanced condition than Thuja, for it appears that only the terminal embryo initial functions. Saxton (36) reported no splitting embryos, but found that several tiers of the proembryo may contribute to the suspensor. However, the existence of cleavage polyembryony may easily have been overlooked.

In Libocedrus, which was studied by Lawson (26), the embryogeny is also too little known to justify any conclusions, and the embryogeny of both Tetraclinis and Libocedrus should be reinvestigated to clarify some of these points.

The Taxodineae and Cupressineae appear to be very similar on the basis of embryogeny, both groups showing an evolution from cleavage polyembryony to simple polyembryony, but our knowledge of both of these groups is at present very unsatisfactory.

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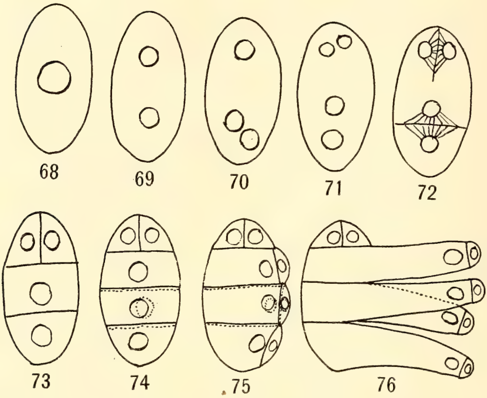
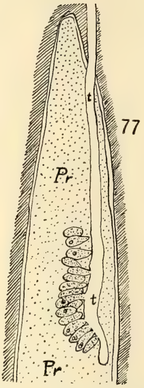
[4] Unpublished work.

ACTINOSTROBUS AND CALLISTRIS

The Actinostrobus and Callitris type of embryo development (figs. 68–76) has certainly been derived from one of the types already described, probably from that of the Cupressineae. In Actinostrobus (35), the first walls appear earlier, between the two- and the four-nucleate stages. The proembryo tissue fills the entire egg, and the separate embryo initials are not organized from the first walled cells, as in Pinus, but after one of the succeeding divisions. Two of the first walled cells undergo no further development, and four embryo initials are organized in the remainder of the egg. Figure 77 shows the relation of the gametophytes and the lateral archegonial complex in Callitris. From this it will be seen that the embryos which grow out laterally from these archegonia penetrate the gametophyte in the usual manner. Saxton, to whom we are indebted for our knowledge of this interesting embryogeny, states that Callitris (34) is very similar, while Widderingtonia (32, 33) is transitional between this and some of the Cupressineae. Cleavage polyembryony is a constant feature, and in all probability the apical cell is also found in the first stages of the embryo.



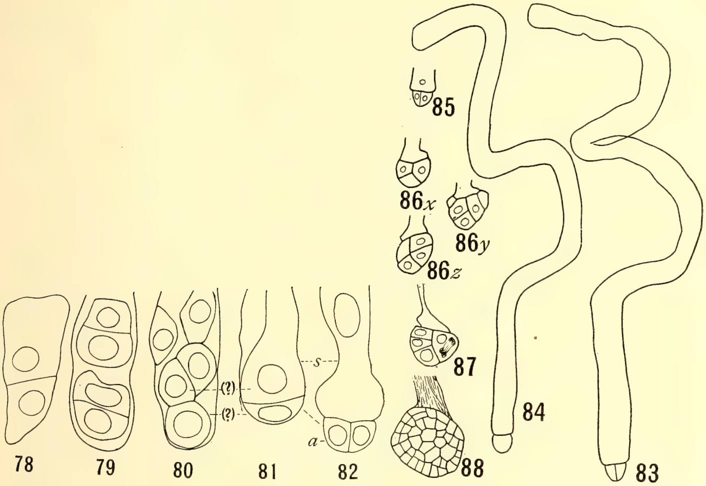


FIGS. 68–76. Diagrams to represent proembryo of Actinostrobus. Callitris, and probably Widderingtonia, are very similar. After Saxton (35). FIG. 77. Gametophytes of Callitris, showing large lateral archegonial complex, in its relation to the pollen tube (*t*). After Saxton (34).

Widderingtonia, Actinostrobus, and Callitris have been placed in a separate sub-family, the Callitroideae, by Saxton (37), largely on the basis of the gametophytic and embryonic characters, and it seems that there is good ground for including these in a group distinct from the other Cupressineae.

SEQUOIA

In the proembryo of Sequoia (23) the tendency to an early wall formation is carried a step further than in the forms previously described, for a wall is laid down after the first division of the egg nucleus (fig. 77). Each of these proembryo cells rounds off more or less, and the cells resulting from the next division are similarly dissociated. According to my interpretation, these cells shown in figures 79 and 80 are separately organized embryo initials, all but the lowest of which abort; at least their further development has not been observed. The lowest cell of figure 79 (or the lowest of figure 80, a point not made clear in the account by Lawson) gives rise to the embryo shown in the succeeding figures, the suspensor cell being the first cell cut off from the embryo initial. This earlier wall formation, the proembryonic tissue filling the entire egg, and the suppression of cleavage polyembryony by the abortion of embryo initials, are some of the advanced embryo characters illustrated by Sequoia.



FIGS. 78–82. Stages in the embryogeny of *Sequoia sempervirens*, × 250, after Lawson (23).

FIGS. 83–88. Later stages in the embryogeny of Sequoia, after Arnoldi (1); figure 87 showing an apical cell. FIGS. 86*x*, *y*, *z*, series of three sections through an eight-celled embryo showing apical cell, after Shaw (38).

There is probably at least a short apical cell stage in Sequoia, according to the figures of Shaw (38) and Arnoldi (1), shown here in figures 83–88.

EPHEDRA

I cannot refrain from including a mention of the Gnetales in this discussion, for these have polyembryony, much as have the Coniferales. In the embryo of Ephedra (22, 40), for example, cleavage polyembryony is the regular occurrence. The free nuclei which are concerned in the organization of the embryo initials do not all descend to the bottom of the egg, as in Pinus, but remain in comparative independence of each other, and this condition of cleavage polyembryony is very striking. Judging from the published accounts and figures there is not much probability that an apical cell stage is to be found, and this would furnish us an instance in which the apical cell is eliminated and cleavage polyembryony is retained. It seems to me that this feature of cleavage polyembryony very definitely marks the Gnetales as having been derived from the conifers rather than from the cycads.

There is perhaps no better way for me to summarize these many comparisons of embryo development made during the course of this discussion, than to use the method of a diagram, as was done for the Abietineae. Such a scheme of phylogeny represents only the present status of our knowledge concerning embryogeny and is not intended to be accurate to the last details, for many of these details have been too inadequately described. It doubtless represents fairly accurately the affinities of the larger groups.

In this diagram (fig. 89), the ranges of the various embryonic features which have been discussed are plotted in light lines, while the (probable) phylogenetic connections suggested by embryogeny are shown in the heavy lines. The range of the apical cell (*A*), and cleavage polyembryony (*Cl.p*) are marked with some uncertainties, but in general it appears that the latter is more limited in its range than is the apical cell. The archegonial complex is found in all forms surrounded by the circle *Cx*. Rosette embryos are not plotted, but the forms having rosette cells are marked as follows: *R*, rosette cells; (*R*), occasional rosette cells; *R-em*, rosette cells that develop embryos; *R*-(*em*), rosette cells that occasionally give rise to embryos, etc. The rosette embryos are therefore also primitive features that soon vanish, followed by the disappearance of the rosette cells, which are the last vestiges of cleavage polyembryony. The embryo cap (*Emb.cap*) found in Araucaria and Agathis is an advanced feature found also in the forms somewhat involved in the evolution of the Araucarineae.

It will be found that in general the cotyledon number decreases as we proceed upward in every direction away from Pinus. Though the number of cotyledons is somewhat variable throughout, this number is reduced sooner or later among conifers (3) to the limit of three or two.

I have included the range of one well-known taxonomic feature in this diagram, namely, the spur shoot (*Sp.S*). Sciadopitys with its double needles in the axil of a bract has doubtless also the morphological equivalent of the spur shoot. If the phylloclad, which occupies the same position on the stem, could be looked upon as the morphological equivalent of this dwarf branch, we should have the dimorphic branches represented in a

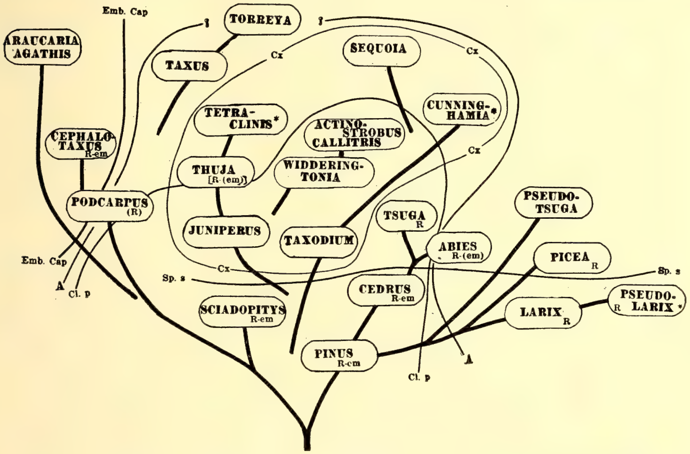

Emb. Cap
TORREYA
?
?
ARAUCARIA
AGATHIS
TAXUS
Cx
SEQUOIA
Cx
TETRA-
CLINIS*
ACTINO-
STROBUS
CALLITRIS
CUNNING-
HAMIA*
CEPHALO-
TAXUS
R-em
Cx
THUJA
[R-(em)]
WIDDERING-
TONIA
PODCARPUS
(R)
PSEUDO-
TSUGA
TSUGA
R
JUNIPERUS
TAXODIUM
ABIES
R-(em)
PICEA
R
Emb. Cap
Sp. s
Cx
Sp. s
A
Cl. p
CEDRUS
R-em
PSEUDO-
LARIX*
R
SCIADOPITYS
R-em
LARIX
R
PINUS
R-em
A
Cl. p

FIG. 89. Diagram representing the affinities of the Coniferales as suggested by embryogeny. *A*, probable range of distribution of apical cell in early embryo; *Cl.p*, probable range of cleavage polyembryony; *Emb.cap*, range of distribution of embryo cap; *Cx*, distribution of archegonial complex; *R*, rosette cells present; *R-em*, rosette cells that usually give rise to rosette embryos; *R-(em)*, rosette cells and rosette embryos occasional only; *Sp.s*, range of distribution of spur shoot. Position of forms marked with asterisk (*) is doubtful.

member of the Podocarpineae. Jeffrey (20) looks upon the spur shoot as a primitive feature, and it would appear at least that this dwarf branch is a character present in the most primitive representatives of more than one line of evolution.

Unfortunately the embryogeny of a large number of conifers is not known, and many forms that have been studied have been described in such a way that we are uncertain of the features with which we are most concerned in the present discussion. Enough has been described to indicate that with some such organization of the facts as here presented, embryogeny must occupy a much more important place in the study of comparative morphology than has generally been conceded.

The origin of cleavage polyembryony, a very interesting question, has not been shown in any living conifer thus far described. A theory to account for the possible origin of this condition has been formulated and is to be published at another time.

SUMMARY

This brief review has summarized, or taken into account, practically all of the published work on the embryogeny of conifers in the proembryo and early embryo stages. It is shown how the known facts may be harmonized under the conception that the Coniferales have been derived from forms with cleavage polyembryony, and that this feature tends to be more or less eliminated as we pass from the lower to the higher forms. The apical cell, cleavage polyembryony, rosette embryos, rosette cells, and the direct organization of embryo initials from the free nuclei of the proembryo, are regarded as primitive features, while the organization of embryo initials after walls form in the proembryo, a proembryo that fills the entire egg with cells, the archegonial complex, the embryo cap, and the return to simple polyembryony, must be regarded as advanced or specialized features. Transitional conditions may also be recognized, and some of the vestigial characters, in disappearing by gradual steps, make this interpretation of the direction of evolution from cleavage polyembryony back to simple polyembryony very certain. Embryogeny must occupy a much more important place in the comparative morphology of conifers than has generally been conceded.

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THE LIVING CYCADS AND THE PHYLOGENY OF SEED PLANTS[1]

Charles J. Chamberlain

Phylogeny is a big word and it can be made to cover most of the problems of relationship. Among the phylogenetic problems of a group, two always stand out prominently: "What has been its origin?" and "Has it left any progeny?"

In the cycads these two problems are not equally difficult, for the origin can be traced back, with more or less certainty, to the ferns; but whether they have left any progeny is doubtful. However, if we stick close to the living cycads, it seems safe to claim that none of the nine genera has left any progeny or is likely to have any descendants. Like the higher members of the Cycadofilicales and Bennettitales, they are the last of their race; and if there should be another epoch succeeding the present, just as the present succeeded the Mesozoic, the Cycadales would become extinct, just as the Bennettitales became extinct.

First let us consider the less difficult problem, the origin of the cycads.

Just how far back the cycads extend, is a question which could be answered only by complete geological evidence; but what we know of available structures shows that the line goes back farther than any fossils yet discovered would indicate.

A morphologist must depend largely upon comparative morphology in studying relationships, tracing each structure, geologically, from its earliest appearance, and tracing the ontogeny wherever material is available.

The graphic method will illustrate clearly some of the principal features in the comparative morphology of cycads and at the same time will indicate their geological distribution (Plate VI).

A very prevalent fern habit—a crown of pinnate leaves at the top of an unbranched stem—has been retained by the cycad line, with remarkable tenacity, from their earliest appearance up to the living forms. The armor of persistent leaf bases is another character which can be traced from the Paleozoic up to the living forms. The large pith, comparatively scanty zone of wood, and large cortex are features common to the living cycads, Bennettitales, and Cycadofilicales (Plate VI).

If these three features—the crown of pinnate leaves, the unbranched stem with its armor of leaf bases, and the topography of a transverse section of the stem—were the only features worth considering, there could be little objection to putting the entire Cycadophyte phylum into one family. But when one turns to the reproductive structures, it is evident that the ancestral stock, the Cycadofilicales, has either differentiated into two lines, or has given rise to the Bennettitales, which, very soon, gave rise to the Cycadales.

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[1] Invitation address read before the joint session of Section G, A. A. A. S., the Botanical Society of America, and the American Phytopathological Society, in the symposium on the "Phylogeny of Seed Plants," at St. Louis, December 30, 1919.

The spore-bearing structures of the Cycadofilicales may be represented diagrammatically: In the center, a crown of much reduced leaves, bearing seeds; just outside these, a crown of reduced leaves—but not so much reduced—bearing microsporangia. But in none of the Paleozoic forms, the Cycadofilicales, is either of these two crowns of reduced spore-bearing leaves compacted into cones. This feature marks the Cycadofilicales, for in the succeeding forms one or both of these crowns of reduced leaves become compacted into cones (Plate VI).

The Bennettitales and Cycadales are best separated from each other by the fact that, in the former, the microsporophylls have not yet been compacted into cones; while in the Cycadales the microsporophylls form closely compacted cones. In both groups, the ovulate structures form cones, except in the genus Cycas.

The microsporophyll is easily traced, not only from the Paleozoic Cycadofilicales, but even from the ferns, up to the living cycads. It was the close resemblance of this microsporophyll to the spore-bearing leaves of Marattiaceous ferns, as well as the close resemblance in vegetative leaves, that led to the earlier geologists to call the Carboniferous "The Age of Ferns."

Throughout the series, the microsporangia are borne on the margin or on the under (abaxial) side of more or less reduced leaves. In the Bennettitales the microsporophylls, while much smaller than the foliage leaves, still show the pinnate character, with no tendency toward becoming compacted into cones. In the Cycadales, the pinnate character has been lost entirely and, in every genus, the compact cone stage has been reached. But the resemblance to a leaf is still seen in the prevailing distribution of the sori into two groups, representing the two series of pinnae, one on each side of a midrib (Plate VI).

The structure of the individual microsporangium has changed very little since the phylum was differentiated from the ferns. It would be interesting to compare the contents of pollen grains of Carboniferous, Mesozoic, and living forms; but no satisfactory fossil material has been sectioned. It seems safe to say that there were no pollen tubes in the carboniferous forms. Engler's term *Siphonogamia* would not include these early seed plants. The small size of the pollen grains, together with the absence of the pollen-tube habit, would indicate that the sperms were very small and that germination and the development of sperms took place very rapidly, as in our living heterosporous ferns.

The immense size of the sperms in the living cycads is an example of giantism which—so paleozoologists tell us—indicates that the phylum has reached its limit and is ready for extinction.

The megasporophyll of Cycas is of the greatest importance in tracing relationships, for it is essentially identical with the megasporophyll of the Paleozoic genus Pecopteris; while in the living cycads, a series of genera like Cycas, Dioon, Macrozamia, and Encephalartos shows the gradual reduction of the individual sporophyll and, at the same time, shows how a loose crown of sporophylls has been compacted into a cone (Plate VI).

This megasporophyll of Cycas is so different from any yet described in the Bennettitales that we think it is safe to claim that the Cycadales have not come from any forms like Cycadeoidea, or from any others with such reduced seed-bearing structures. While we should recognize the phenomenon known as atavism, or reversion, we believe it could appear only after a rather limited time. We can easily believe that a Pecopteris-like megasporophyll has persisted from the Paleozoic up to the present time; but we could not believe that the megasporophyll of Cycadeoidea, if reduced from a Pecopteris type, could—after millions of years—revert to the Pecopteris type, and so give rise to a megasporophyll like that of Cycas. We might believe in spontaneous generation and in the special creation of species, but not in that.

Consequently, if the Cycadales are a branch from the Bennettitales, the point of union is so far back that it becomes a question of arbitrary definition rather than a question of fact whether there has been a main stock with an early branch, or whether there have been simply two lines coming independently from the Cycadofilicales.

This seems to me to answer the question, "What was the origin of the living Cycads?" as far as it can be answered in the present state of our knowledge. If Professor Wieland would give us three big books on the Cycadales of the Triassic, Jurassic, and Cretaceous, like his three big books on the Bennettitales, we could state facts instead of spinning theories.

In tracing the plane body, with its stem, leaves, and spore-producing structures, from the Paleozoic up to the living cycads, the record is fairly complete, and there is not a very serious danger of mistakes; but in tracing the origin of the seed the Cycadophyte line has afforded little evidence, for the seeds—as far as they have been described—are almost as highly developed in the Paleozoic as they are today. In this line, they must have come from heterosporous ferns. But, until some one finds and sections a convincing series in heterosporous ferns, or in some more primitive members of the Cycadofilicales than any yet discovered, we must base our theories of the origin of the seed upon the behavior of living heterosporous forms which have not quite reached the seed stage.

What is the answer to the second question, "Have the Cycads left any progeny?"

Something has left some progeny; for an abundant progeny, both Angiosperm and Gymnosperm, is very visible and very much alive. What groups could have been responsible for this progeny?

If we consider only the nine genera of living cycads, as we know them today, the answer is easy: they are not responsible; they are the last of their race, restricted in geographical distribution, restricted in numbers, and struggling for their very existence.

To some this may seem like too positive a statement; but if it should be challenged, we should ask, "To what could the cycads have given rise?" The only possibilities are the Cordaitales, Ginkgoales, Coniferales, Gnetales, and the Angiosperms.

The Cordaitales, as the ancestral stock of the Coniferophyte line, might be expected to show resemblances, if any were to be found; but in habit they are very different from the Cycadophytes. They are the forest types, while the Cycadophytes bore somewhat the same relation to them that the ferns of today bear to the forests in which they occur. The leaves are prevailingly simple, contrasting sharply with the prevailing pinnate or twice pinnate leaves of the Cycadophytes. Not enough is known of spore-producing members in the Cordaitales, to make safe comparisons, but the Cordaitales certainly had well-developed cones; so that, in this feature, they had progressed far beyond the Cycadofilicales. The fact that the cones were compound, while those developed later in the Cycadophyte line were simple, would indicate that the Cordaitales were from a different stock. We believe the available evidence indicates that the Cordaitales have come directly from the Pteridophytes; but whether they have come from the fern section or from the lycopod section is a problem in the solution of which morphological characters of still undetermined value are balanced against each other.

In the Ginkgoales, the pollen-tube structures, with the two motile sperms, present a startling resemblance to the corresponding structures in the cycads, even to the blepharoplasts developing into spiral ciliated bands, the peculiar behavior of the persistent prothallial cell, and the haustorial habit of the pollen tube. The extensive free nuclear period in the development of an embryo with two cotyledons is common to the cycads and Ginkgo; but here the resemblance ceases. The plant body and the strobili make relationship seem impossible. As far as the Mesozoic cycads are known, they afford no better Ginkgo resemblances.

In my opinion the Bennettitales are no more nearly related, although I once tried to compare the long-stalked ovules of Ginkgo with the ovulate strobilus of the Cycadeoidea type.

Even if we go back to the Paleozoic Cycadofilicales, it seems no easier to establish a relationship. Besides, the Ginkgoales can be accounted for quite naturally as an offshoot from ancient Cordaitales stock.

A relationship with any of the Coniferales would be even more difficult to establish. Corresponding structures are too contradictory. The large pinnate leaves of the Cycadophyte line do not compare well with the small, entire leaves of the Coniferophytes; nor does the unbranched trunk of the former compare well with the profusely branched trunk of the pines and Ginkgo.

In trying to provide progeny for the Cycadophytes, some have cast a hopeful eye upon the Gnetales, because the staminate flower of Welwitschia has a sterile ovule and thus presents a bisporangiate condition in which a vivid imagination might see some resemblance to the bisporangiate strobili of the Bennettitales. But my imagination is too weak to see more than a superficial resemblance, even in this feature; while a comparison of the stems of the two phyla, the comparison of pinnate leaves with simple leaves, and of simple strobili with compound strobili, seems impossible.

Could the Cycadophytes have given rise to the Angiosperms?

For the living cycads, we should answer with a positive *no*. This conclusion cannot be escaped, if we compare the haustorial pollen tube and its contents with the sperm-carrying pollen tube of the Angiosperms. The large, ciliated, highly differentiated sperms of the cycads are headed for extinction rather than for evolution into the comparatively simple structures of the Angiosperms. The extensive free nuclear period in the development of the cycad embryo does not compare well with the total lack of such a period in the Angiosperms. However, reductions in the free nuclear period are not entirely impossible.

It is true that the general habit of the cycad, with its unbranched stem and crown of pinnate leaves which form an armor of leaf bases, is so strongly suggestive of palms that the layman calls Encephalartos the "Bread palm," Dioon the "Dolores palm," Cycas the "Sago palm," etc. But the resemblance is superficial. A section of the palm stem shows an advanced monocotyl condition, and the flower is truly monocotyl. It may seem like begging the question to say that the Monocotyls have come from the Dicotyls, but we believe this has been proved as definitely as anything is likely to be proved in relationships.

The resemblance between the Bennettitales and the Angiosperms is about the same; but here an attempt has been made to reconcile the floral structures. The resemblance pointed out was between the Bennettitales flower and a sympetalous flower. Our objection here would be along the same line: the sympetalous condition is a modification of the polypetalous, and the Sympetalae, like the Monocots, have come from the Archichlamydeae.

In the Cycadofilicales we are nearer the source of things, but the discrepancies keep becoming greater and greater and indicate that we are on the wrong trail. Like the hasty student, trying to pigeon-hole *Eryngium yuccaefolium* among the Monocots, we need to go back and make a fresh start.

We have tried to show that the Cycadophytes have come from the ferns, by way of the Cycadofilicales directly or as an early branch from the Bennettitales; and we have also tried to show that they have not given rise to any other seed plants.

This might seem like a logical place to stop, for we have tried to answer our two questions: "What was the origin of the cycads?" and "Have the cycads left any progeny?"

But it would emphasize the answer to the second question if we could show that the visible progeny could be referred to some other ancestry. In case of murder, the victim constitutes a concrete fact to be accounted for. The defendant may claim he didn't do it; but it adds weight to his claim if he can cast suspicion on some one else.

So let us ask another question: "Could some other group have given rise to the Coniferophytes and the Angiosperms"? We shall consider the two groups separately.

If the Coniferophytes have not come from Cycadophytes, they must have come from the ferns or from the lycopods. This is a problem, in the discussion of which leaf gaps are balanced against leaves, pinnate leaves against simple leaves, and abaxial sporangia against adaxial. I believe the evidence is sufficient to establish a Pteridophyte origin; but the comparative claims of ferns and lycopods do not appear the same to me as they did several years ago.

As far as the seed is concerned, some of the Paleozoic lycopods, like some of their living descendants, had progressed so far that their megasporangia are separated from seeds by arbitrary definitions rather than by facts.

We separate the Gymnosperms from the Angiosperms by the ovules on open carpels and ovules enclosed in an ovary; and the distinction is good and very useful in a taxonomic key; but rigid definitions may harden our ideas and may prevent us from getting an unbiased view of the facts.

In most Angiosperms, except epigynous forms, the ovules appear on open carpels, the closed ovary developing later. In cases like the Ranunculaceae, the integuments of the ovule appear and the embryo sac is well started while the carpel is just as open as in any Gymnosperm. In the Amentiferae, the ovules are well started before the carpels close; and in Podophyllum, sometimes the carpels do not close at all, the ovules being borne on perfectly open carpels, as in the Gymnosperms.

In considering this whole subject, we must remember that the extinct forms which have been preserved are mostly woody, especially in the Mesozoic. Has there been an extensive herbaceous flora which has disappeared? Have we lost herbaceous Gymnosperms which may have given rise to herbaceous Angiosperms? And could such herbaceous Angiosperms have given rise to the woody Angiosperms which became prominent in the Cretaceous?

Unless such an herbaceous flora has arisen and disappeared, it is necessary to derive the Cretaceous Angiosperms from woody forms; and this means from more or less well known Cycadophytes or Coniferophytes. Such attempts have been made. We have already paid some attention to the claims of the Cycadophyte line.

In looking for the origin of the Angiosperms, the claims of the Coniferales and the Gnetales may be considered separately, although they have much in common.

Familiar representatives of the Coniferales show more resemblances and fewer contradictions. The plant body is similar, and the internal structure of the stem often shows striking resemblances. The catkins of the Amentiferae may not differ much, morphologically, from some of the cones of the Coniferales; the pollen-tube structures of Angiosperms could be derived from those of Coniferales, and the embryogeny could be reconciled. The leaves are harder to reconcile, but leaves are very susceptible to environment.

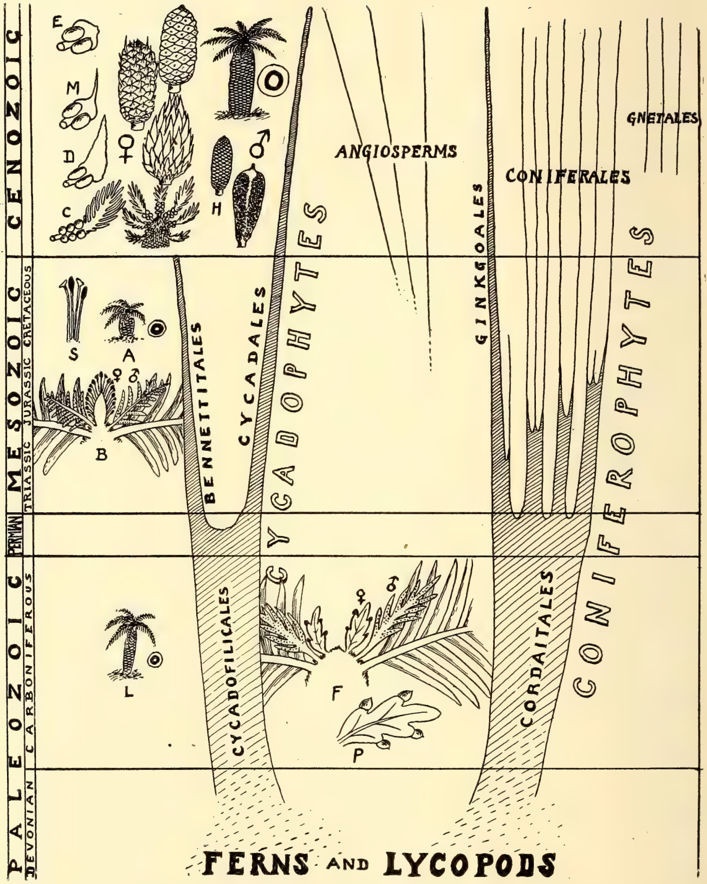
While these resemblances would not induce us to claim any Coniferales yet described as the ancestors of the Angiosperms, we believe they indicate the direction of the trail. We should remember that most of our Paleozoic and Mesozoic material is woody, and that there is a possibility—I believe there is a strong probability—that a great herbaceous vegetation has failed to be preserved, or, at least, has not yet been discovered. In such an herbaceous vegetation, leading up to woody forms, I believe the missing links will be secured, and that the Angiosperms will be found to extend much farther back than any available records have indicated.

The Gnetales show some striking Angiosperm characters. Most botanists, looking at the habit and leaves of *Gnetum Gnemon*, would call it a Dicot, and the histology of the stem continues the Dicot impression. In Ephedra, the habit, the strobili, and the spermatogenesis show Angiosperm features. It is so evident that the leaves have been reduced from more pretentious structures, that they need not constitute any objection.

In this connection, the less said about the leaves and habit of *Welwitschia*, the better; but its flowers, especially the staminate flower with its sterile ovule, would pass for Angiosperm flowers. The only objection seems to be that definition relating to open and closed carpels. Fortunately we have reached a stage in botanical development at which definitions need not interfere with research; for we do not put the Liliaceous Agapanthus in the Dicots simply because it has two cotyledons; or Nelumbo into the Monocots because it has only one cotyledon. So the open and closed carpel need not be absolute marks separating all Gymnosperms from all Angiosperms, and the presence of one condition or the other need not interfere with research into the origin of the Angiosperms.

It is easy to be humorous and to say that an ancestor must be older than the offspring, and that, therefore, the Gnetales, with no geological record, could not qualify as progenitors of anything. But here, again, we must remember the possibility, or probability, of an extinct herbaceous flora, which, very late in its history, developed a few woody members. Earlier in its history, it may have given rise to herbaceous Gnetales and to primitive Angiosperms, which developed into the woody forms of the Cretaceous.

We have tried to show that the Cycadophytes have come from the ferns and that they have not left any progeny, outside of the Cycadophyte line;


CENOZOIC
MESOZOIC
TRIASSIC
JURASSIC
CRETACEOUS
PERMIAN
PALEOZOIC
CARBONIFEROUS
DEVONIAN
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D
C
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O
♂
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S
A
♀ ♂
B
L
F
P
BENNETTITALES
CYCADALES
CYCADOPHYTES
CYCADOFILICALES
ANGIOSPERMS
GINKGOALES
CONIFERALES
GNETALES
CONIFEROPHYTES
CORDAITALES
FERNS AND LYCOPODS

Chamberlain: Cycads and the phylogeny of seed plants.

and we have tried to emphasize the second claim by showing that the Coniferophytes and Angiosperms—the undoubted progeny of something—can be referred to another ancestry.

EXPLANATION OF PLATE VI

Diagram illustrating some features of the Cycadophytes and Coniferophytes.

At the bottom: *L*, a diagrammatic representation of a member of the Cycadofilicales, with a transverse section of the stem at the right; *F*, an idealistic view of spore-producing members; *P*, pinnule of *Pecopteris* with seeds on the margin.

In the middle: *A*, habit of one of the Bennettitales with section of stem; *B*, bisporangiate strobilus; *S*, two seeds on long stalks and two scales.

At the top: habit of a living cycad; *C*, sporophyll of *Cycas* with crown of sporophylls at the right; *D*, *Dioon; M*, *Macrozamia; E*, *Encephalartos;* each with corresponding cones at right; *H*, male cone with a single sporophyll below. All very diagrammatic.

DISTRIBUTION AND RELATIONSHIPS OF THE CYCADEOIDS[1]

G. R. Wieland

I. Distribution

Plant geography is an impressive subject. It should find extension in time. Hitherto, little more than the fossil plant localities have been indicated. But the larger outlines of the Mesozoic forests must yet appear. The characteristic forms are slowly being determined; and sufficient progress has been made in paleogeography to permit initial hypothetic mapping of some of the forests. That even this rougher mapping discloses new facts is certain. With the old continental boundaries in view it becomes logical to ask why the Rhaetic plants of the Virginia-North Carolina coal field are so megaphyllous, while those of the southern Andine region are very microphyllous. Does not a larger part of the Jurassic Ginkgo record also indicate wide climatic variation, second only in extent to that of the time of the Glossopteris flora? Would it not be singular if plant evidence remained wholly at variance from that of the insects and invertebrates, indicating climatic cooling in the late Trias and early Jura, not local in character?

When one-sided evidence is once recognized as such, it becomes less misleading. The picture of the typical Mesozoic forest with a tropic sun beating on its xerophylls has been too grandly simple. A remnant of the equisetes, ferns, Araucarias, cycads, the pines, and the Ginkgos! Think this over. No real forests except coniferous "pure stands" from the close of the Permian to the Comanchean angiosperms? Unbelievable. The evidence already carries us much further, and the fact is being slowly disclosed that varied forests of microphyllous cycadeoids must have had a greater area than all other gymnospermous forests put together, all through Triassic and Jurassic time.

The record is not scanty, as I know from the field. There has been no reason for the view that the fossil cycads are simply the underbrush of tropical forests, or were merely columnar-stemmed fringing types like the palmetto. Yet this has been the only view. Nathorst, indeed, left open the question of the habitus of Wielandiella; but Jeffrey thought this form was procumbent. Williamsoniella (see fig. 1) would look less so. There is, however, no evidence for procumbency in either case. On the contrary, the branching in both these small-stemmed cycadeoids is but little simpler than that of some magnolias, and it is easier far to look upon them as shrubs, or as trees with a habitus not unlike some of the araucarians, the Brazil pine for instance. The point is all but proven, despite the fact that the actual histologic structure awaits fortunate discovery. By any fair analogy the pith must be little or no more developed than in young magnolia shoots, or in cone-bearing branches of araucarians; while the wood structure could not have been very different from the cycadeoidean type. Furthermore, along with the small branches go small leaves, and the small perfect flowers, just as suggestive of forest types as those of the tulip tree. Evidence fails

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[1] Invitation address read before the joint session of Section G, A. A. A. S., the Botanical Society of America, and the American Phytopathological Society, in the symposium on the "Phylogeny of Seed Plants," at St. Louis, December 30, 1919.

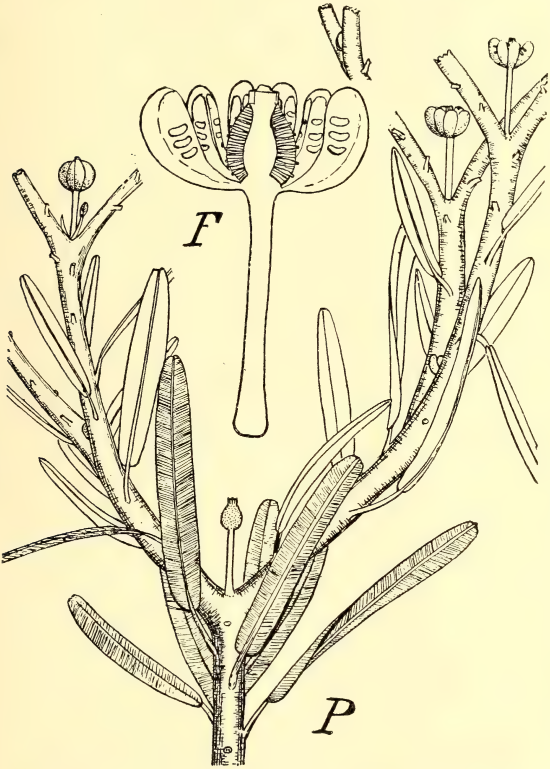


FIG. 1. *Williamsoniella coronata*, from the mid-estuarine series of the mid-Jurassic of the Yorkshire coast (at Gristhorpe). From the restoration of Hamshaw-Thomas. *P*, branch-end with flower-buds; *F*, a single flower enlarged twice. The central cone of the flower is surrounded by a whorl of synangia-bearing scales.

for the view that the fertile dichotomizing branch ends, all thus far found, are anything else than the broken-off branches of trees. Just such branches of conifers of like or of older age are found. The chances that these forms had either structures or a habitus in any way indicating procumbency, are exceedingly small. In groups or in forests they might have had some likeness to screw poines of mountainous rather than of tropic rain forest districts. The peculiar Pandanus forest of the Lokon of the Celebes is here suggested. But the point to note first is, that there is the fullest reason to believe that the small Taeniopteroid leaves of the small-flowered cycadeoids are related to innumerable megaphyllous types of truly tropic habitat, only the latter would be less plastic forest elements.

Whether this view of relative aplasticity for these megaphylls is right or wrong, they soon undergo extinction, after in their prime vividly recalling in both habitat and habitus the earlier coal-swamp floras. Neither Williamsoniella nor Wielandiella taken by itself indicates tropic plants at all. They were probably tropophylls or plants which shed their leaves with the seasons. The stems are usually found bare, the attachment of the dissociated leaves being determined only with difficulty. These are in a word generalized plants which so far as habitus goes might well grow in temperate to cool climates. Until far more is learned about them they should at least be held valueless as indices of tropic climates. But as the small-stemmed cycadeoids were related to the contemporaneous Ginkgos, and at the same time to early angiosperms, the inference becomes direct that either they or their close relatives already had the capacity to live in every clime.

There is also a suspicion that study of the associated ferns may compel revision of the long-accepted view of the universality of tropic climates throughout the Mesozoic. A. G. Nathorst, the most eminent living student of fossil plants, says of these suggestions in a letter just received: "I think you are quite right that during the time when the Ginkgophytes and Cycadophytes dominated, many of them must have adapted themselves for living in cold climates also. Of this I have not the least doubt. Remember, for instance, *Juniperus communis*. If Juniperus were extinct, and conclusions were drawn from all the other species found fossil in the parallels where they now live, it would be believed that the whole genus was bound to live only in the temperate climates. Yet *Juniperus communis* thrives well in Greenland."

Since current opinions of Mesozoic climates as based on plants are so open to challenge, any details which can possibly be learned about the cycadeoid distribution have a doubly important bearing on phylogeny. But it must be freely admitted that the subject can only be approached slowly, and is here considered superficially.

In a fossil form distribution is, to use a long and emphatic word, bidimensional. Distribution in a living form is simply lateral; but in fossils it is both lateral and vertical with more or less uncertainty at all limits.

As a rule, more is known of the vertical range or persistence in geologic time than can possibly be learned of the lateral range for a given period. And in nearly all fossils the probable period of extinction is more determinable than the first appearance. This follows for several citable reasons, and especially in the case of plants. Nearly everything, moreover, depends on the habitus of the plant, and upon where it grew. Generally the three thousand species of coal plants appear cosmopolitan because in the Carboniferous certain coastal plains were peculiarly favorable places for conservation, and now the economic value of coal so abundantly laid down leads to vast excavation over hundreds of square miles of the rocky strata, and through thousands of feet in thickness. How different is the case where some Permian, Rhaetic, or mid-Triassic horizon is studied. The excavation for material then depends on the enthusiasm of about a dozen men, taking the world over. This explains almost in a word why the record in the Carboniferous seems extensive, and in later periods much scantier.

It has long been held that cycads or Cycadophytes, as now more broadly named, dominated the Jurassic especially. But probably botanists, who have outnumbered paleobotanists a hundred to one, have generally been taken aback on noting that the score or more of well-marked post-Carboniferous floras seldom include more than 100 species in all. And on comparing, for instance, the Liassic of Scandinavia, England, India, and Mexico, it is even more disquieting to find that the species look stereotyped, as if they belonged to a few nearly related groups and gave but a vague picture of contemporaneous vegetation. But here the graver difficulties end. Except in the case of the Mesozoic gymnosperm stems, vast in quantity, of rare beauty of conservation, and urgently demanding study, the paleobotanist quite invariably deals with larger features. Just as the microscope reveals histologic detail, so separation in time magnifies structural and other changes to the point of visibility.

Thus far there appears to be no great fallacy in taking the cycadeoids from a generalized point of view and by percentages observing their ratio of abundance to the other forms of the successive horizons. This is in effect a rough consensus of plant life taken from age to age. The results are of course open to different interpretations, and it is most difficult to draw lines between all of the greater groups. In going back there is a gradual mergence of Coniferophyte, Cycadophyte, and Ginkgophyte foliage toward the seed-bearing quasi-ferns, at once indeterminate and startling to observe. Then very far toward the early Paleozoic there seems to be some kind of contact between the early seed ferns, and the older Lepidophyte types also leading toward the primitive gymnosperms. As to whether, well down in the Devonian, some of the Lepidophytes of the Pseudobornia alliance were in near contact with Archeopteris, and like the later seed ferns also led into the primitive Coniferophytes, is the real sphinx riddle of paleobotany—far more so than the origin of the angiosperms. It looks as if plant life would have been best balanced in the phytologic sense if the Devonian Pteridophytes and Lepidophytes both sent their quota into the gymnosperm complex. In that case some Cordaites (?), araucarians, and perhaps some other Coniferophytes would be these Lepidophyte derivatives.

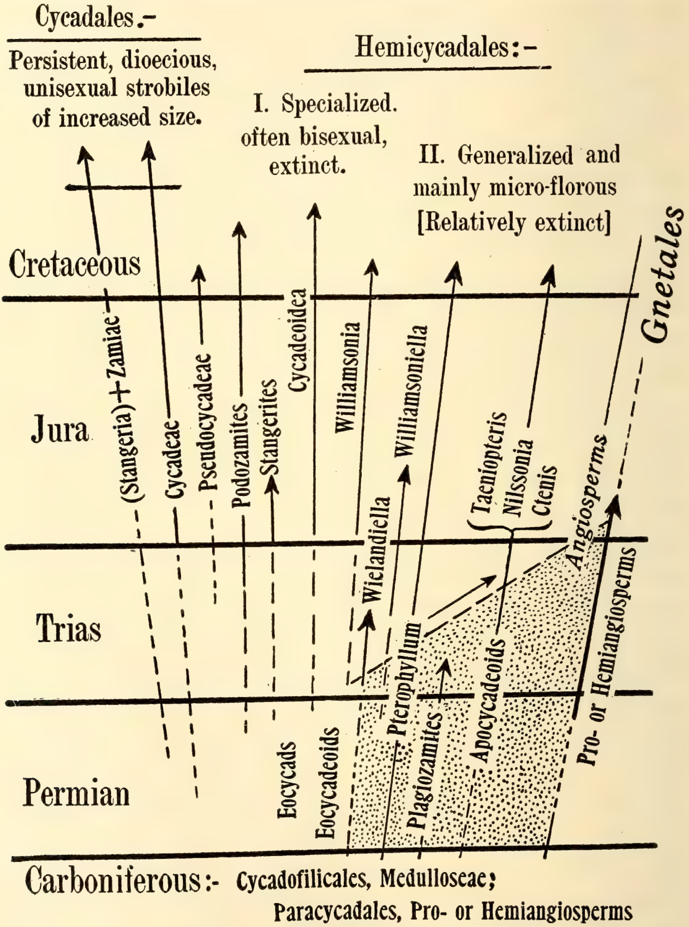

Cycadales.-
Persistent, dioecious, unisexual strobiles of increased size.
Hemicycadales:-
I. Specialized. often bisexual, extinct.
II. Generalized and mainly micro-florous [Relatively extinct]
Gnetales
Cretaceous
Jura
Trias
Permian
(Stangeria)+Zamiae
Cycadeae
Pseudocycadeae
Podozamites
Stangerites
Cycadeoidea
Williamsonia
Williamsoniella
Wielandiella
Taeniopteris
Nilssonia
Ctenis
Angiosperms
Angiosperms
Pro- or Hemiangiosperms
Pterophyllum
Plagiozamites
Apocycadeoids
Eocycads
Eocycadeoids
Carboniferous:- Cycadofilicales, Medulloseae;
Paracycadales, Pro- or Hemiangiosperms

FIG. 2. Diagrammatic scheme showing the position in geologic time of primitive and hypothetic groups related to the cycads and cycadeoids, and their assumed relation to the basal angiosperm types. The position of the latter is indicated by the stippled area.

Bearing in mind, then, these greatest of all problems of plant history, many of the genera so difficult to place could be cited. A few may suffice. Podozamites lies on the cycadeoid-conifer boundary. Brachyphyllum stems must approach in structure as well as in appearance the cycadeoid stem types. But yet, by constant and consistent attention to main phases of the fossil plant alignment, and by continual revision of the percentual record of occurrence, the general nature of the forward movement of cycadeous plants can undoubtedly be brought out. It is very interesting indeed to find that in the Australian Rhaetic and Lias the proportions of the several plant phyla are in harmony with those noted elsewhere. In fact, there are certain features of Mesozoic vegetation which stand out as very important, and which could not be discerned without the aid of this percentual method. It displays the great abundance of the Pterophyllums at the earlier end of the cycadeoid record. Also, the Taeniopterids which are separated from the other forms by A. B. Walkom in his Australian studies, as they should be, are a singularly prevalent type in the late Triassic. They are often small-leaved, and if largely cycadeoid they are of course the forms which stood very close to the leaf types leading toward the dogbanes, the oleanders, and the Magnolias. In the Lias, where climatic variation is suspected, the stereotyped pinnate fronds of the tropics (Oaxaca) mark the culmination of plants apparently cycadeoid.

As an example of distribution and relative abundance expressed percentually, the subjoined table (table 1) from Walkom may be scanned. It displays the relations found in the plants of the "Ipswich series" of the lower Mesozoic rocks of Queensland, Australia.

TABLE 1.

	No. of Species	%	%	%
Equisetales	5	15	15	15
Filicales	10	30		39
Filicales (*incertae sedis*)	3	9	57	
Tæniopteris	6	18		
Cycadophyta	2	6	6	24
Ginkgoales	7	21	21	21
Total	33			

Such tables are certainly helpful, with the fossils actually in hand. And their graphic value can scarcely be denied. As Walkom observes, "they must be used with a good deal of caution, lest they lead to quite incorrect and even absurd results; although with a full realization of their value and also their drawbacks, they may yield interesting and to some extent reliable results." Note that the Equisetales suggest a Triassic abundance, while the considerable number of Filicales, with a large (early) gymnosperm series, is in accord. The general description of the flora given by Walkom sustains his conclusion that these plants may be of upper Triassic (or

Keuper) age. But in the original list there are four species of Thinnfeldia, which are probably ginkgoid, and if so considered would reduce the Filicales to a more normal proportion. Further tabulations of Mesozoic plants may be found in Volume 2 of my "American Fossil Cycads."

As in the case of the Dinosaurs, the cycadeoids after they reach relatively high specialization, move rapidly toward extinction during the phase of continental development which begins with the great epeiric seas of the upper Cretaceous submergence and ends in the full continental areas of the glacial stages and later or present arid climates. This is the period, not of angiosperm origins, but of angiosperm dispersal and specific modification with disappearance also of the early or transition angiosperms.

One other observation, and the subject of distribution may be left aside, it hardly being practicable to go into moot questions of generic distribution for the moment. In almost all instances the doubtful border of cycadeoid foliage ends in a tree forest of seed ferns, Cordaites, pines, Araucarias, and Ginkgos, but never in recognizable scrub. With the legitimate inferences from stem structure, and the characters of Wielandiella, and especially of Williamsoniella in mind, a much greater Mesozoic forest comes into view. Nothing in paleobotany appears more probable now than that amongst the cycadeoids will be found the lost forests and the greatest forest makers of the Mesozoic.

II. Relationships

If the systematist can recognize a degree of relationship or similarity between the monocot arums and screw pines, and the Ranalean dicots, why is not oblique or inequal convergence the more difficult explanation? Those resemblances must have been still greater in the Jurassic forest. But even then these several lines must have been distinct. Nothing has so limited progress in phylogeny as the *potting* of "paleontologic trees." If more attention were given to the elementary facts of the record as found, progress in its interpretation would be surer. For whether, in that lofty mood, variation is held to be epigenetic, or orthogenetic, or whether it be held that there is less of continuity and that the main course of biologic change goes on in select lines and types with much outright extinction, both the object and the method of phylogenetic study remains the same. The primal object is to determine the order in which structures and organs appear, and thus to find how the groups of animals and plants are related in time. From any more philosophic viewpoint classifications are only made to serve this purpose, and thus afford a sound basis for the more ultimate study of variation. And therefore, while classification is at every stage in the development of plant study a serious task, classifications themselves should be viewed as wholly impermanent. As a definition of classification, then, may be given, simply, *present views of relationship*.

In attempting to elucidate some of the principles which must influence our views of the relationships of the cycadeoids, and in assembling the broader known facts and passing on to some quite legitimate inferences, the present object is of course to bring into view mainly those features which have a bearing on the phylogeny of seed plants in general. In the glimpse just had of distribution, attention was mainly fixed on relationships within the Cycadophytes. It was found that no headway could be made in picturing the real extent of cycadeous vegetation in the Mesozoic, without some consideration of the hypothetic variation within the group. And that subject could have been pursued much further. Now it is the aim to single out analogies without the group. Being essentially gymnosperms, it will be contended that the cycadeoids relate themselves to the other spermatophytes in the following order of closeness: firstly to the cycads, secondly to the seed-bearing quasi-ferns, thirdly to the Cordaites and Dolerophyllum, fourthly to the Ginkgophytes, fifthly to Araucaria, sixthly to the Abietineans, seventhly to the magnolias and other dicotyls, and eighthly to the Gnetales. This order may be conveniently followed in discussion.

The Cycads

There has been a wide divergence of opinion as to whether the cycadeoids are in any near sense related to the cycads at all. But as knowledge of the existent and extinct groups has been extended, and as better defined terms have been reached, the difference of opinion or of viewpoint is lessened. So distinctly is this true that it would hardly be fair to name any one, either in this country or in Europe, as holding unqualified views. One might say that the likenesses between the two groups are distinct and the differences striking, or the reverse. And this alternative or disposition of some to lay stress on vegetative features in this classification, and of others to emphasize fructification, has found expression in the division of the supergroup Cycadophyta into the Cycadales and the Hemicycadales or half-cycads. Certainly no one would deny that the cycads and cycadeoids are the two most contiguous of the greater gymnosperm phyla. The two groups must have come from the same section of the Carboniferous plant alignment. Throughout all of Permian and Triassic time they must have been in close histologic contact, and by lower Jurassic time about all the visible difference in the wood was the preponderance of scalariform wood in the cycadeoids in contrast to the pitted wood of the present-day cycads. Both wood types occur in both groups, and histologists are welcome to think as they please about which is the more primitive. Of course, while insisting upon points of vegetative resemblance it is the large pith and thin woody cylinder of the petrified stems, or the family Cycadeoideæ, which is cited. But the fact cannot be too strongly emphasized that such stems are of unusual type. They are the only ones definitely known amongst the cycads. It was seen, however, that the characteristic and plastic cycadeoids were no doubt small-stemmed and microphyllous. The single strand leaf trace appears rather primitive as compared with the double strand of the cycads, and may have had some relation to microphylly and plasticity of type. But the double strand appears in ancient gymnosperms; and, also, in Ginkgo the leaf traces arise from the stele as a pair of collateral bundles. Nor would it be cause for surprise to find in some small-leaved cycadeoid with a thin cortex such a double trace, or even two weak lateral traces.

Turning to fructification, the contrast between the two types is great because of sporophyll emplacement coupled with retention of the primitive microsporophyll in one instance and a carpophyll in the other. But the cycadeoid microsporophyll was also plastic and reduced in well attested instances from both the Triassic and Jurassic rocks. It might, therefore, be believed that some members of the original cycadeoid alliance had both the mega- and microsporophylls reduced in spiral emplacement. Such, however, would lead toward Gnetalean or coniferous types, and what appears to have been an instance will presently be cited. It may be added that the observation that the Cycadeoidea microsporophyll was as distinctly horned or bicornute as that of the Mexican Ceratozamia, and freely tomentose, brings the groups together a bit, and at the same time suggests possible form variation toward conifers.

The Seed Ferns

Derivation of the Cycadophyta in totality from ferns is in accord with the views held by botanists throughout all the studies of existent and fossil plants for the past two or three score years. This is a section of botanical science to which its votaries may point with confidence if not with pride; and further discoveries are awaited with the certainty that they will be made. The seed fern Lyginopteris was fully hypothesized before its final discovery. But adequately to treat this antecedent relationship would require an attention to structural details beyond the limits of the present discussion. It is safe to say that both the vegetative and the reproductive hiatus between the quasi-ferns and the early cycadeoids is bridged by known structures, found isolated to be sure, though conclusive. One of my colleagues has also given consideration to this fundamental relationship.

The Cordaites and Dolerophyllum

The origin of the Cordaiteans is so lost in geologic antiquity that an otherwise rather striking affinity is more or less obscured. It must be remembered, too, that in going back so far toward the beginnings of these plants with seeds often of enormous size, the likenesses must frequently be the merest of parallelisms. Could sessions like ours have been held about the close of the Devonian, when *Callixylon Oweni* flourished where we now stand, it may well be imagined that discussion would have turned on whether the Cordaites seed was phyletically related to the synchronous ancestral cycadeoid seed or not.

The singular Dolerophyllum is another type very difficult to place, but with a seed fern antecedent in Pecopteris (fig. 3). The linear spirally inserted leaves are not unlike those of the Cordaites. The large pollen believed to pertain to these leaves is borne on a large, fleshy, peltate disc 6 by 5 cm. in a series of very elongate pockets more or less regularly radiating from the eccentric insertion. Whether these pockets are rows of more or less confluent sori or synangia is not clear, but possible, since vascular strands run between them. If the disc were symmetrical, or could it be shown to arise from fusion in a whorl of fertile leaflets, affinity to the staminate discs of Gnetum and the cycadeoids would be foreshadowed. Somewhat similar discs are seen in the Neuropterids called Potoniea and Linopteris, also in

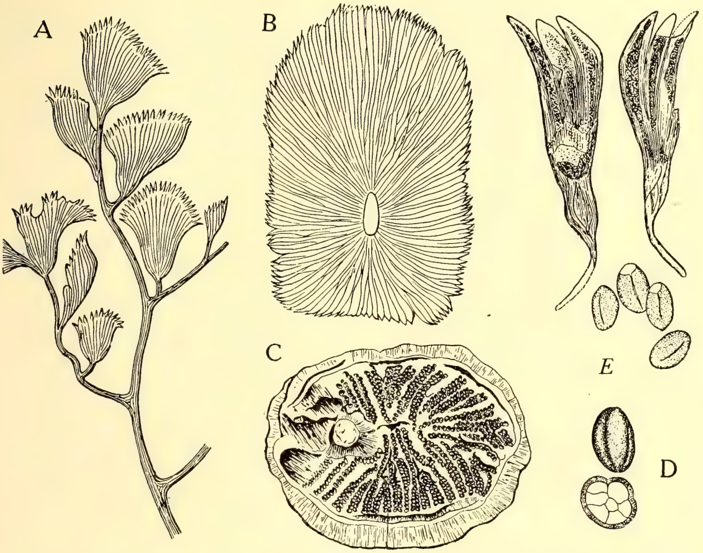


FIG. 3. Ancient staminate disks: *A*, *Potoniea adiantiformis* of the French Carboniferous, a Neuropterid bearing staminate cups (natural size); *B*, *Linopteris antiqua*, also of the French Carboniferous, showing under side of disk (several times enlarged); *C*, Dolerophyllum, a fleshy staminate cup (with pollen enlarged at *D*); *E*, *Codonotheca caduca*, showing the microspores (enlarged), and the toothed and symmetric campanula (natural size) from the Carboniferous of Mazon Creek, Illinois. *A* and *B* from Bertrand, *C* and *D* from Renault (Seward), *E* from Sellards.

Note. The microspores of Ceratozamia are 40 microns long, those of Cycadeoidea 50 to 100, of Stephanospermum 120, of Codonotheca 300, and those of Dolerophyllum 400 microns long. All the evidence thus far tends to indicate that ancient microspores were large.

Neuropteris Carpentieri of Kidston. The completely symmetrical disc Codonotheca, an abundant and striking fossil in the coal measure nodules of Mazon Creek, Illinois,[2] also appears to fall within this Neuropterid-Dolerophyllum alliance. It is believable that the study of these ancient discs must eventually show the manner of evolution of the large vascular gymnosperm seeds; unless indeed the synangial hypothesis of Professor Margaret J. Benson for the origin of seeds accounts for the sole method.

With this brief mention Dolerophyllum may for the present be dismissed. As a floral type it finds place somewhere amongst the Medullosans, an immense assemblage of Paleozoic stems structurally antecedent to those of the Mesozoic and later Cycadophyta. Unfortunately, fructification in this group, though not entirely hypothetic, is about the blackest *incognito* of paleobotany.

The Ginkgos

A great Ginkgophyte phylum, falling but little later in geologic time, next arrests attention. The Ginkgos are mainly Permo-Jurassic, and especially exemplify the fact that the forward movement in plant evolution was always widespread, with the higher of the extinct forms of the successive periods always holding near to the persisting mean. Specialization in the Ginkgos seems to rise little beyond oddity of outer feature. Berry mentions as members of this phylum, Ginkgophyllum, Saporteae, Whittleseya, Trichopitys, Dicranophyllum, Rhipidopsis (cf. fig. 4), Psygmophyllum, Gomphostrobus, Tricophyllum, Feildenia, Phoenicopsis. There are also the handsome leaves called Baiera, with the staminate flowers, or Antho-

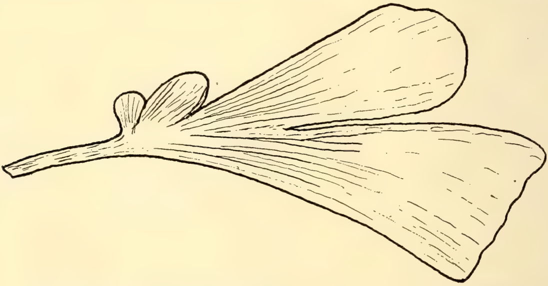


FIG. 4. Rhipidopsis, a ginkgoid leaf which occurs in the Permian of India and Russia, and which strongly suggests relationship to the South American fronds mentioned in the text as typical in the Rhaetic. Only the outline is shown. This remarkable foliage type was described by Schmalhausen, and a photograph showing the typical Ginkgo venation is given by Seward in his "Fossil Plants," volume 4. Here only half the natural size.

---

[2] On the split surface of a Mazon Creek nodule of my own, no larger than the palm of the hand, four complete Codonotheca discs appear, and there are parts of a fifth and sixth. They seem to have split off regularly, like fronds.

lithus, possibly known. Some of the foliage no doubt falls near or within the Medullosans. But other forms may be added. Of such the lax cone Beania with two-seeded megasporophylls denotes variation toward the cycadeoids, so distinctly contemporaneous in the Trias-Jura transition or Rhaetic time. Here also I would mention the two remarkable Y-branched frond types known as Dicroidium and Thinnfeldia, both recently shown by Anteys to be xerophytic. They have been referred at one time or another to several gymnosperm groups, but not hitherto to the Ginkgos. In any case the fernlike aspect relates these frond genera on the seed-fern side. Nor do they appear remote from that somewhat more cycadeoid leaf type called Ptilozamites. This genus and the palpably ginkgoid and varied Baiera foliage, occur in well marked association in the Rhaetic of the southern hemisphere. No one who studies the Rhaetic and the succeeding Liassic or lower Jurassic plants in the field will ever again rest under any doubt about a steady and well marked transition from seed fern foliage toward cycadeoid and ginkgoid foliage. With this point emphasized it may be permissible to omit closer reference to structure, and to ask attention to a cycadeoid relationship of a more recondite character because of a certain lack in the accumulation of fossil evidence, namely that to Araucaria.

Araucaria

That the araucarians attained specialization early, with retention of much primitiveness of feature, and that they are a discrete line coming down from the old cycadofilicalean complex, is indicated by analogy to the cycadeoids. It is now evident that Araucaria has more in common with cycads and cycadeoids than was earlier supposed. The robust armored stem is analogous to that of the cycadeoids, this being true of structure, of cortical development, and of both the primary and the secondary branching. The roots freely send up young plants, and the seedlings are stout, cycad-like, and remarkably tenacious of life. Renewed growth of the reproductive shoot from a lateral bud is cycadaceous and cycadeoid, comparison being made with Wielandiella and *Williamsonia scotica*. The large pith and thin woody cylinder of the shoots, vegetative and reproductive, and the complete transition from foliage to fertile scales of the large cones are also cycad-like, as well as still more decidedly cycadeoid. The megasporophyll with its small ligule finds a counterpart in the decurved microsporophyll of the cycadeoids, and is aplosporophyllous, with the seed imbedded.

Nor is it necessary to regard the araucarian seed-cone as greatly different from that of Cycadeoidea merely because the seed is decurved like that of cycads, instead of erect. In reality the fertile sporophyll is surrounded by infertile members almost identically as in Cycadeoidea. This significant comparison has been hitherto overlooked. Moreover the araucarian microsporophyll is also decurved and at the same time sends up an acuminate scale-tip which may well be regarded as the analogue of the spur seen in the cycadeoid microsporophyll. In fact, if the latter is reduced, as it may be, and then imagined to be spirally inserted as in forms already hypothesized, the main features of the araucarian staminate cone appear to view. Finally, the presence of a leaf gap opposite the outgoing foliar trace in the stem and seedling adds still more weight to this far-reaching comparison. The double and multiple traces do not of course compare directly with the single trace of the known cycadeoids, but with cordaiteans or cycadeans. But some or all of the resemblances or parallelisms pointed out must have been more marked in the Jura. The araucarians have probably simplified more or less since then, in accord with their simple foliage type and narrowing distribution.

Pines, and Gymnosperm Stem Structure

Amongst gymnosperms the pines of today are of course the type remotest from the cyadeoids; but so far as may be judged from the lax or less compacted, even leafy, types of gymnosperm cones which prevail in the late Paleozoic and early Mesozoic, there may be hypothesized a marked similarity between some of the ancestral pines and the cycadeoids. This general subject is a most difficult one, and adequate study of the abundant gymnosperm stems in most fresh-water deposits of the globe from the Paleozoic down has never been made. Obviously such work can be pursued only by the most expert students of wood structure. Enough has been done, however, to lead to the belief that tracheidal change has followed some fixed trend, just as has floral change.

Bailey and Tupper have examined the size variation in tracheary elements of the secondary wood of vascular cryptogams, gymnosperms, and angiosperms. It is positive that there has been much decrease in tracheidal lengths since the evolution of the upper Devonian Cordaite forest, and in widely separated groups. Also, Willis and DeVries have observed a tendency of plants to present certain features and groupings or segregations, which persist or fail over wide areas. There is a tendency to division into "locals" and "wides" which leads to a belief in some ratio of age to area. The theory alone is in a sense self-destructive. If changes in secondary wood are progressive through the ages, and if in the more superficial characters of leaf and flower the vegetation of forest and plain is still subject to simultaneous change, there is no such thing as *age* and *area*. One form is about as old as another. But right or wrong, the contributions cited taken in combination with the work of Clements on "plant succession," form the chief current contribution by botanists to the broader study of evolutionary theory.[3]

---

[3] Digressing a bit: Such coordinated change went on amongst the wonderfully patterned ammonites all over the globe all through the Jurassic. And why not? R. A. Harper says: "From the one-celled alga or fungus to the highest plant or animal, the differentiation of nucleus, cytoplasm, chromosomes, spindle fibers, etc., is everywhere present; and in their general nature and functions and in their interrelations, these structures are the same.

The general subject of later tracheidal structures as bearing on the origin of modern stem types is too broad to take up in any detail. But a few observations may be made. There are a number of facts accessible especially in the great work of Solereder, going to show that no one process can account for the origin of vessels. Possibly they have at times arisen by direct evolution very anciently in unknown and upland Arctic floras, and later secondarily from both pitted and scalariform tracheids. Perhaps, as Jeffrey contends, scalariform wood can even result mainly from pit fusion. But it will not do to call only pitted wood ancient, and the scalariform types the more modern. The remarkable Carboniferous Lyginopteris has the large-celled, many-pitted wood, but either the contemporaneous relatives or the ancestral types of the quasi-ferns may and must have had the scalariform wood. The peduncular wood of cycads and cvcadeoids alike is scalariform.

This much may be safely said: In the pines a high degree of ray specialization is geologically recent. Also in the dicotyls the course of ray change must be coordinated with recent development of storage tissues. Such structures may be subtracted in order to glimpse or to hypothesize antecedent dicotyl wood in the Jurassic. If then the pit wood of Drimys and Trochodendron with its suppressed growth rings, and the scalariform wood of Trochodendron and Tetracentron, have any signficance at all, the inescapable conclusion is that both cycadeoid and cycad wood is old and near the type basal to many modern forms. It is indeed delusive to read this history in terms of Gnetum alone.

Dicotyls and Gnetaleans

Analogies, rather than relationships, between the cycadeoids and the dicotyls and gnetaleans may be quite conveniently discussed as under a single topic. For here the gymnosperm border line is crossed, and all the near relationships cease. The older view of dicotyl derivation through early conifers and gnetaleans is now opposed to the newer view of near cycadeoid derivation, in part coupled with suggestions of an extreme parallelism amongst both gymnosperms and angiosperms. But following various recent and thorough studies of the gnetaleans, the idea that they indicate the real angiosperm precursors is even accentuated by some. Lignier and Tison say the gnetaleans are merely aberrant angiosperms which retain early gymnosperm features and lead toward the amentifers. And Hallier even suggests they are reduced dicotyls like Loranthus and the Myxodendraceae.

E. W. Berry is the most recent to follow and emphasize the Lignier and Tison view, so far as relates to descent. He says that the primitiveness of

. . . Evolution has not consisted in the production of new types of protoplasmic structure or cellular organization, but in the development of constantly greater specialization and division of labor between larger and larger groups of cells."

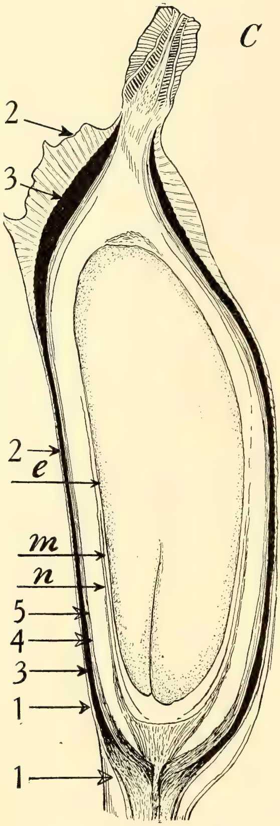


FIG. 5. *Cycadeoidea Dartoni* seed in longitudinal section (radial to cone). *1*, *1*, termination of tubular celled cortex forming supporting basal cup to erect seed; *2*, *2*, the blow-off layer of radially set cells enveloping shoulder region much as in *Gnetum Gnemon; 3*, *3*, lateral and shoulder development of the radially celled stony layer which is prominently four- or five-ribbed; *4*, a thinner schlerenchyma of small elongate cells or fibrous hypoderm; *5*, main inner parenchymatous layer; *n*, the nucellus arising from the chalazal base containing central supply of numerous small scalariform tracheids; *m*, megaspore membrane; *e*, dicotyledonous and exalbuminous embryo filling the nucellar cavity. Length of seed *four millimeters*.

the magnolia flowers is illusory, and he finds reasons for dicotyl derivation from the Gnetales in: (1) the inflorescence, (2) floral morphology, (3) the details of sporogenesis, (4) fertilization, (5) embryogeny, (6) organization of vessels in the wood, (7) broad rays, (8) companion cells in the bast, (9) habit and foliage, and (10) the dicotyledonous embryo.

This is a sweeping summation, so regardless of plant history that it would scarcely be expected to come from a paleobotanist. It does not set aside the possibility that the gnetaleans have merely paralleled the angiosperms, as Seward and others have suggested. In any sense of finality in evidence, the validity of these points falls one by one. It will not do to compare the gnetaleans of today with the cycadeoids of the Trias. And what the gnetaleans were like in the Jura, the fertile time of angiosperm origin, is too uncertain. Neither can 20,000 to 30,000 species be safely hypothesized for them as in the case of the cycadeoids. They can not be hypothesized out of hand on vague leaf characters. Commenting, then, *seriatim* on these "points": It must be insisted that taking the greatly reduced Wielandiella flower of the Trias, nothing is simpler than to infer related forms with few-seeded flowers grouped spirally. It is a mistake to attach all significance to this mere sporophyll emplacement, or to relation between singly borne and inflorescent flowers. This might arise late or early. Nextly, it is wrong from even the purely histologic standpoint to assume that the Gnetum flower of the Jurassic was more reduced than cycadeoid flowers. Besides, though separated by such a great lapse of time, the Gnetum and cycadeoid seeds show some peculiar resemblances pointed out by Emily M. Berridge and Mrs. Thoday (see fig. 5). As Seward well says, it would be "rash" to hold such resemblances without phylogenetic significance. So also the details of sporogenesis may merely tend to parallel those of angiosperms, and may thus be deceptive—illusory, as Berry thinks the primitiveness of the magnoliaceous flower to be. Nor is it necessary to assume that none of the cycadeoids advanced beyond a motile antherozoid stage. This view I was quite the first to put forward strongly, and must retract. The negative view alone is permissible as a hypothesis.[4]

In the embryogeny is perhaps found the very strongest evidence for dicotyl derivation from gnetaleans. W. P. Thompson observes much similarity, and some differences which may yet prove fundamental; but the subject is discussed by one of my colleagues.

So far as regards the gnetalean wood, it must be urged once more that the vessels have been held to have peculiarities, and that the extent of parallel development since the Jurassic cannot yet be fairly estimated. W. P. Thompson says the vessels "should be removed from all discussions of the angiosperms." If so, then, similarly, the rays. The foliage of *Gnetum Gnemon* is of a peculiar netted type with a striking fineness of mesh not so very dissimilar from that of the laurel-leaf magnolia. Netting, however, probably developed progressively in the seed plants, and could as readily accentuate in pinnate cycadeoid blades, either primitively or secondarily netted. If the net is primitive in Gnetum, it can be primitive in the cycadeoids. If it resulted from separation, or alternant elision of the pinnate veins, with invasion of the marginal net in an earlier Gnetum, leading towards oleanders and magnolias, the same development could go on in cycadeoids. There, too, a real basal form is recognized in the fern-like Taeniopterid leaves of the flower-bearing Williamsoniella. That net venation was very anciently and widely present in the Cycadophytes is indicated by the fern-like mesh in the pinnules of the Indian Dictyozamites, one of the stereotyped Liassic cycadeoids.

To continue, W. P. Thompson, in concluding one of his studies of gnetaleans, quotes an abbreviated statement of Scott on the "claim" of a cycadeoid-angiosperm ancestry as resting simply on three points—strobilar organization, fruit-enclosed seeds, and the exalbuminous nature of these.

---

[4] Stefanie Herzfeld emphasizes my own observation of conductive nucellar tissue in Cycadeoidea as evidence of zooidogamic fertilization. And that this mode was formerly more or less widespread amongst the gymnosperms must be believed. Evidently, then, there is need to have a care in excluding such a mode from the cycadeoids. But it may be noted that the exact comparative study bearing on this point is scarcely made, while the object here is mainly to state the case theoretically. The zooidogamic type of fertilization must have disappeared mostly as the modern angiosperms arose, or mainly in the interim between the Rhaetic and the Cretaceous. So that in this time of great change amongst the cycadeoids as well it seems unlikely that they continued more primitive in this respect than conifers.

Then Thompson goes on to say that the negating cycadeoid features are the cycadean habit and leaves, motile spermatozoids (!), the primitive gymnosperm condition, and absence of angiosperm adumbration in the gametes, endosperm, or embryo. Thompson as a botanist pays even less attention to chronology in his assemblage of characters than does Berry as a paleobotanist. A cycadeous habit for Williamsoniella or Wielandiella! Never, if the thought is only of gnetaleans! And, of course, if fossil foliage is to be excluded from the reckoning, what should be done with Tumboa? Leaf variation is not a special feature of gnetaleans. Besides, as they still persist, they probably changed late or more or less inadaptively, and too slow to be ancestral to anything.

Those wishing to examine the gnetaleans from the critical phylogenetic point of view should begin with the work of Lignier and Tison. It is briefly excerpted and commented on in my "American Fossil Cycads," volume 2, pages 235–237. In their summary of the features of the hypothetic Gnetaloid precursor of the angiosperms may be discerned a fundamental type which could not have been remote from some of the contemporaneous cycadeoids. The great question remains, at what period did the main separation actually begin? When this becomes even approximately known, intensive search may be made for the fossil evidence and field relations.

If allowed a subscription of faith, if permitted a prediction, then I make mine that future work will develop the fact that plant evolution has followed an orderly sequence and course. Its current has been as sure, as steady, as that of the majestic river by the banks of which we stand. From age to age the great groups have come down side by side, some specializing certain features a little more, others holding to more generalized structures, or losing apparent relationships because of reductions, but all undergoing that endless change from which neither genus nor species has ever been exempt. Almost no forms, scarcely a family, need be regarded as more ancient or more modern than any other.

Huxley, with his keen insight, noted as a most astonishing thing the fact that, taking all animal life, the proportion of extinct ordinal types is so exceedingly small. In the 125 orders of animals only about ten percent, perhaps now fifteen percent, appeared wholly extinct. But with all the advances made in paleozoology revealing complexity of form, there has been much of simplification, and type after type has been found much older than at first thought.

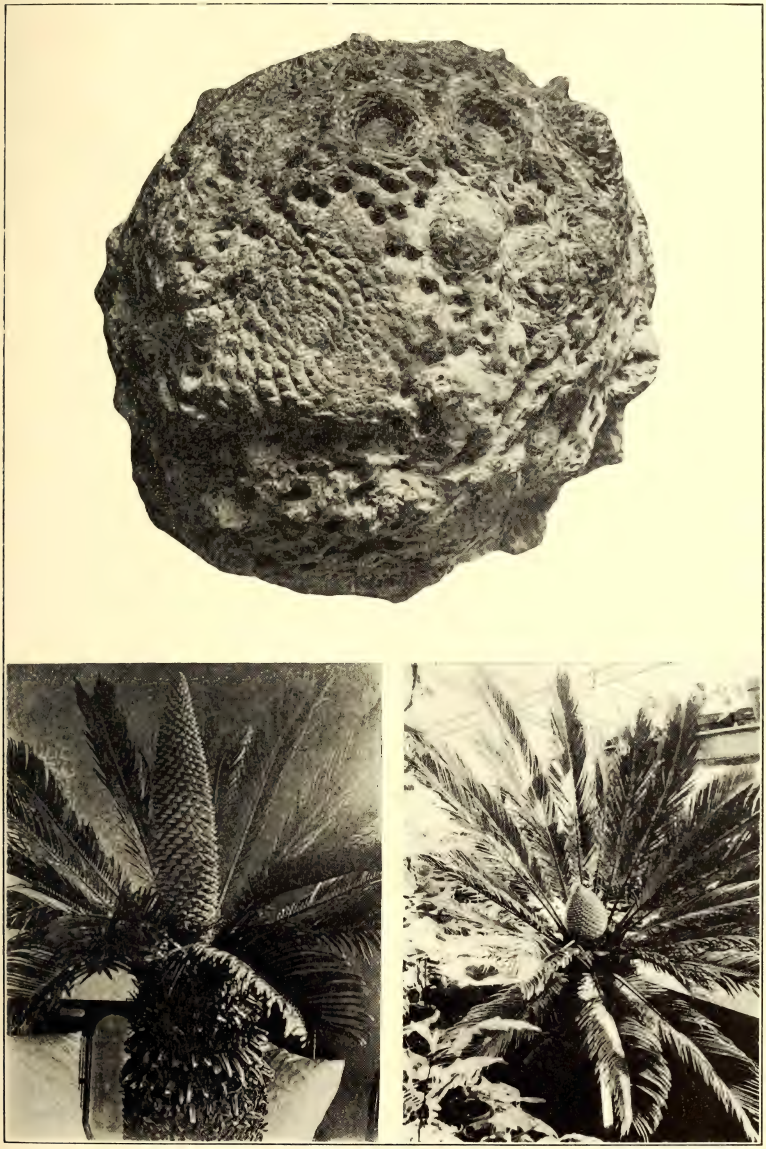
The plant record is, so far as the higher types are concerned, both older and more fragmentary than is much of the animal record. Its study has been late in development, and has often lagged. The results from the different continents are as yet poorly coordinated. Nevertheless the broader outlines of ancient vegetation already appear. The known gymnosperms and the pseudo-gymnosperms or cycadeoids go back to the Paleozoic, and it is conceivable that all the antecedent types of the angiosperms are equally discrete, always separate lines, leading back to the first forests that clothed the land in the Devonian. And throughout all later time it may well be believed that with the poles where they now are, and with that tremendous rhythmic diastrophism or emergence and subsidence of the continents, there was an ever-present plasticity in the plants of the arctic areas. But along with the theory of hardiness and invasive power for the plants of the high north and far south would perforce go a similar potentiality in plateau and mountain vegetation.

EXPLANATION OF PLATE VII

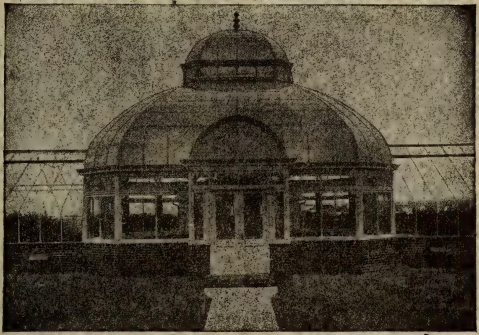
Above: Apical view of *Cycadeoidea dacotensis* (type) showing terminal helicoid of young chaff, enveloped fronds, and scale leaves, with various fruit buds, about ¼ natural size.

Below: *Cycas revoluta* (left), showing cone about 15 inches high, and (right) the same in a younger stage of growth.

These figures illustrate extreme cycadophytan types.



WIELAND: THE CYCADEOIDS.



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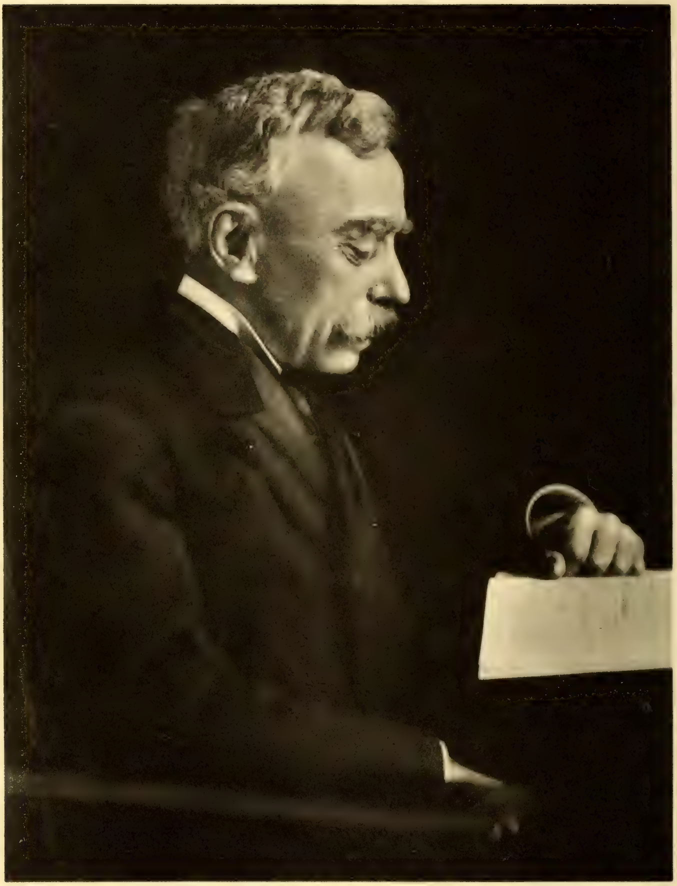

Yours, sincerely, W. G. Farlow

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WILLIAM GILSON FARLOW[1]

December 17, 1844–June 3, 1919

A. F. BLAKESLEE, ROLAND THAXTER, AND WILLIAM TRELEASE

Since the death of Asa Gray, in 1887, no American botanist has been accorded quite the esteem in which Professor Farlow was held. As with Bornet, this was due even more to a large influential acquaintance and a recognized conservative well-informed sanity in judgment, than to volume or importance of the publications of his later life. Indeed, for some years past he has been rather hesitant about putting into print things that he knew better than others—possibly through the realization of age, that nothing is really finished even when an expert gets to the end of what he can do with it; that sometime or other somebody else can go as far; and that in any event somebody else will have to start again at the beginning, sometime or other.

Merely to possess a large acquaintanceship does not mean necessarily that a man will be liked or admired or respected. Professor Farlow's personality was such that with few and unimportant exceptions the many who had the privilege of knowing him liked and admired and respected him to an unusual degree. His character and talent and learning were such as to command affection, admiration, and respect. If either attribute was ever withheld by a colleague or acquaintance it was because of an utter failure to understand his nature, which did not court praise or deference and sometimes in an effort to escape one or the other prompted a seeming cynicism or levity which was as unreal as it was ready and brilliant.

Dr. Farlow was characterized not only by an artistic temperament but by unusual quickness of perception and response. Those who knew him best were likely to hesitate before engaging him in even the most friendly of bantering encounters: but his tongue was not sharp for those of whom he disapproved, and when he wanted to bring a thing into question he had the art of doing it by some most inoffensive but nevertheless unmistakable anecdote or figure of speech.

Men who enlist the interest of others differ greatly in the way in which they communicate their own enthusiasm. Gray bubbled over with it as he worked and talked. Farlow was much less effusive, but those who were

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privileged to know him and study under his supervision saw his manifest interest in their work and imbibed some of his unobtrusive enthusiasm over his own work. It is unlikely that any student who came near enough to him in space or age or mentality really to know him, remembers him more vividly in any respect than as a kind friend—observant, thoughtful, and helpful, but with a tact that prevented any impression that he saw the need of the help that he gave.

In the history of American botany, Professor Farlow figures as the personality through whom thallophytes passed into the field of college botany. Classic work had been done on them by men not filling college chairs, and voluminous work of lasting value continues to be done by such men: but it was his privilege to teach as well as to investigate in this field. He considered himself a botanist rather than a phycologist or a mycologist, and he never called himself a phytopathologist.

Many of his published papers deal with the algae, and his opinion on our seaweeds was taken everywhere and always as authoritative; but he did not train many men in their study. When his own opportunity to work under a master came, it was the fungi that he elected, and De Bary to whom he went; and his greatest service as a teacher and an investigator was rendered in this special field of botany, out of which the half-segregated practical applications of plant pathology evolved during his lifetime.

Though never very robust, and subject to frequent distressing if not serious ailments, Dr. Farlow was an indefatigable worker and an insatiable reader—never satisfied with what somebody said that somebody else had said. During the later years of his life he was freed from the burden of teaching, but compelled to shoulder a business responsibility involving the administration of large financial interests. He neither had nor apparently wished the relaxation commonly considered the due of a septuagenarian. Like the friend and mentor of his youth, Asa Gray, he died in the harness; and the great herbarium and library that he has left to Harvard University with a liberal endowment will keep in memory the debt of his Alma Mater and of the botanical world to him, our foremost authority on the thallophytes, as effectively as the greatest student of American flowering plants is commemorated in the Gray Herbarium of the same institution.

The traits which marked Professor Farlow's mature and professional life were forecast in his descent and development. He was born and educated in Boston. His parents were of New England stock, and his father in addition to being a successful business man was active in public service and a supporter of horticultural and musical organizations. As a student he was as brilliantly diverting as his intimates found him to be in later life, with a penchant for natural history. He graduated from Harvard College in 1866 and from the Harvard Medical School in 1870, and for the next two years assisted Professor Gray in the botanical department of the college. The next two years were spent in Europe, partly in travel and partly as a graduate student at Strasbourg. For several years after his return home he taught in the Bussey Institution of Harvard University as assistant professor of botany; but from 1879 until his retirement from active service in 1896 he taught in Cambridge, with the title of professor of cryptogamic botany, which he held up to the time of his death, when he had become the senior member of the faculty.

Publications

The following list of Dr. Farlow's publications has been prepared from memoranda furnished by Mr. A. P. D. Piguet and is as nearly complete as it has been possible to make it, except that none of his numerous reviews of books and articles have been included.

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Montia sibirica in Massachusetts. Rhodora **18**: 240.

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THE DEVELOPMENT OF THE THALLUS OF SPHAEROCARPOS DONNELLII AUST.

H. W. Rickett

The Germination of the Spore

Previous descriptions of the germination of the spore of Sphaerocarpos represent two conflicting views. One of these is held by Leitgeb and Goebel, the opposing one by Campbell. These are the only authors who have described spore germination in Sphaerocarpos. Leitgeb (9), the first to study this subject, identifies the method of spore germination with that typical for the Ricciaceae and Marchantiaceae. There is, according to him, a germ tube formed, consisting at first of a single cell, and later of several tiers of cells arranged in groups of four. The terminal quadrant of this structure forms the germinal disc, and one of its cells becomes the apical cell of the thallus. The latter grows out in a plane at right angles to the long axis of the germ tube. The apical cell is at first two-sided, cutting off two sets of lateral segments; it is later replaced by a four-sided apical cell, forming dorsal and ventral segments in addition to the lateral ones, and thus causing the thickening of the thallus. The first rhizoid is formed very early, but he states that its connection with the sporeling was to him obscure, though obviously arising from a previous division of the original cell.

Campbell (3), studying spore germination in Riccia, was unable to confirm this account; according to him growth is continuous in one direction throughout the history of the sporeling, and there is no formation of a germinal disc or plate on the end of, and at right angles to, the germ tube. According to his description, the germ tube, consisting of several tiers of cells, is formed as described by Leitgeb, and from one of the terminal cells a two-sided apical cell, later replaced by a four-sided cell, is formed; but growth resulting from the segmentation of this apical cell takes place in the same direction as does that of the germ tube. He agrees with Leitgeb in classifying Sphaerocarpos with the typical Ricciaceae and Marchantiaceae so far as the method of spore germination is concerned, but differs from him in his description of the method by which this takes place. In his account of the process in Sphaerocarpos (2), he describes in detail the way in which the two-sided apical cell is replaced by a four-sided cell. The two-sided cell is divided by a basal (posterior) wall in a vertical transverse direction, and then begins to cut off three sets of segments instead of two, two lateral and one basal. Later, two sets of basal segments are formed, the one basal wall being replaced by two inclined to one another. Thus finally four sets of segments are formed, two lateral, one dorsal-posterior, and one ventral-posterior. He gives no details as to his method of studying this transformation. Campbell also states that the first rhizoid grows from the basal cell of the germ tube near the spore wall. It is not usually formed until the young plant is multicellular, and it is not separated from the germ tube by a wall. Later rhizoids arise from the older cells of the young thallus.

Goebel's description (7) in general confirms that of Leitgeb. According to him, however, it is impossible to say that the thallus develops from a single cell of the terminal quadrant of the germ tube, which cell functions as an apical cell from the beginning. On the contrary, all the cells of this germinal disc take part in the growth of the thallus, an apical cell is discernible only at a later stage, and one cannot determine from which cell of the germinal disc it was developed. He tends to bring together the two opposing views of Leitgeb and Campbell by calling attention to the fact that the formation of the germinal disc is not the development of a new structure from a sort of protonema, but is rather a simple flattening out of the young thallus presumably as a response to external conditions of air and light. The chief evidence in favor of this idea is found in a comparison with the method of growth in regeneration, which may resemble either description of the method of spore germination. He contrasts this type of development, which he calls "homoblastic," with that of the "heteroblastic" sort, in which the body of the plant arises secondarily from some sort of protonema.

A difference in species may perhaps account for the difference in descriptions. Campbell, according to Miss Haynes (8), was working with either (or both) *S. texanus* or *S. cristatus*. The European authors probably studied *S. Michelii* and *S. texanus*. It is also, of course, not inconceivable that differences in climate and in general habit might influence the course of development.

The description which follows is based on a study of sporelings of *S. Donnellii* grown from spores sown broadcast in a mixture of clay loam and sand and kept in a Wardian case in the greenhouse. When the young plants became visible under a hand lens as minute, bright green growths on the surface of the soil, they were picked up with a needle under a binocular microscope, the soil was washed off in water, and the plants were mounted in glycerin. A solution of chrom-acetic acid (chromic acid, 0.3 g.; glacial acetic acid, 0.7 cc.; distilled water, 99 cc.) proved most satisfactory for fixing. Various attempts were made to stain the sporelings, but without much success. They took the stain with difficulty, and it did not prove permanent. However, since in most cases the cells were slightly plasmolyzed, the cell walls stood out fairly sharply although they did not always maintain exactly the original form of the cells.

Different attempts were made to germinate spores in culture solution, using that recommended by Marchal and Marchal (10), but it was impossible to keep the cultures free from fungi brought in on the spores or on small shreds of tissue. In some cases germination occurred, after about twice the normal period, and resulted in the production of some abnormal plants. I was unable to grow any of these plants to maturity, on account of the fungous growth which soon covered and choked them.

The spores sown in soil germinated usually about a month after sowing, in the case of those sown early in the spring. Some sown in early summer required three or four months to germinate. The first sign of germination is the appearance of a slender germ tube which pushes out through an irregular ruptured spot in the heavy spore wall. The spores from a greenhouse culture are at this time still united in tetrads, which is contrary to the usual description for *S. Donnellii* under natural conditions (8). The spores of a tetrad do not usually all germinate at the same time, and it is rare to find a tetrad with all four spores producing young plants. The germ tube is filled with dense cytoplasm, and contains from the first an abundance of chlorophyll, and there are often present in the early stages globular bodies having the appearance of oil droplets. As the tube grows in length, the dense contents become gathered at the distal end, leaving the basal end almost clear and quite colorless, and the first wall usually cuts off a terminal cell which contains all, or nearly all, of the dense cell contents. The length to which the germ tube may grow before the first cell division occurs varies considerably. This is evident from a comparison of figures 1 and 2, Plate IX. In cases in which I have made measurements, the length of the undivided germ tube varies from 0.3 to 1.6 mm. The majority of the germ tubes reach a length of about 0.5 mm. by the time of the first cell division. In the cases of the abnormal sporelings mentioned above which resulted from spores germinated in nutrient solution, the most remarkable feature was the great length to which the germ tube attained. It seemed to be able to grow indefinitely until the distal end reached the surface of the solution, at which time cell division usually occurred (fig. 35, Pl. X). Some that I have measured reached a length of 7 or 8 cm., and consisted of a single long hair-like cell with a small mass of dense green material in the extreme distal end, and only to be distinguished by this latter characteristic from the rhizoids produced at the same time.

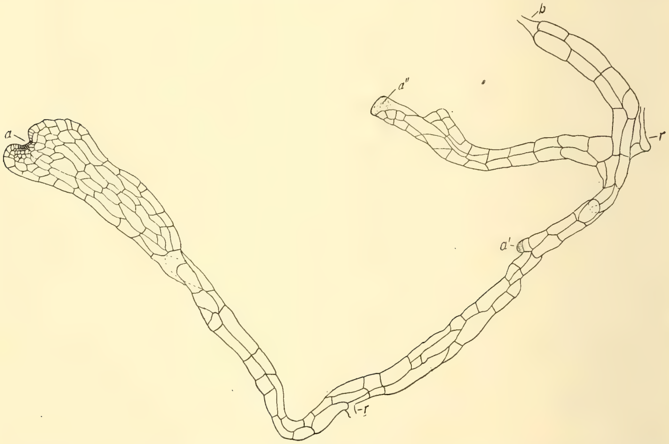
The first wall is transverse, and divides the germ tube into a large clear basal cell, from which only the first rhizoid develops and which undergoes no further division, and a small, densely-filled terminal cell from which the rest of the young thallus develops (fig. 2). The basal cell persists for some time, so that the young plant remains attached to the spore wall until well along in its development—until about the time when the secondary disc begins to be composed of two cell layers.

The second cell division occurs in a plane parallel to the first, cutting the terminal cell into two (fig. 3). About this time the first rhizoid usually appears, pushing out from the extreme base of the germ tube, and growing rapidly in the opposite direction. It is a simple slender structure, almost devoid of granular contents except at the extreme tip.

The next division is variable, and indeed the whole subsequent history, up to the time when the young thallus has been fully formed, is very inconstant. Figures 4 to 9 indicate some of the methods by which the development occurs. The end cell resulting from the second transverse division may divide longitudinally (fig. 4), or other transverse walls may first be put in, and any of the resulting cells may divide longitudinally (figs. 5, 7). The result is usually the same in all cases—namely, the formation of a small plate of cells (commonly six or eight) of about equal size arranged in pairs (figs. 6, 8). Occasionally, however, some of the longitudinal divisions may fail to occur, the result being a single cell at some point in the double row of cells in place of a pair of cells (figs. 7, 9, 11). The next divisions are also longitudinal, but at right angles to the preceding, the result being a series of several groups of four cells each, with, however, many departures from absolute regularity. Very frequently the groups consist of three cells instead of four. The arrangement of cells here is difficult to determine under the microscope, as dividing walls are often either in the plane of the slide or superimposed vertically one upon the other. Figures 13, 20, 21, and 23 represent the arrangement of the cells at this time. These figures are of older stages in the development of the thallus, but all the cells except those of the terminal group have remained undivided.

The cells of the end group now begin to divide, mostly by longitudinal walls, so that a plate of small cells is formed at the end of the germ tube (figs. 10–16). These divisions are apparently more rapid than those preceding, since the cells do not attain so large a size before redividing. The divisions seem to be quite irregular, and I failed to find any cell in these stages which could be recognized as an apical cell. In figures 10 and 12 there is shown a cell (*a*) cut off by a diagonal wall suggestive of the apical cell described for many liverworts; this appearance, however, is not the usual one. The typical appearance of the young thalli at this stage is best shown in figure 13. The groups of cells below the terminal plate undergo no further divisions, though rhizoids are often produced from them; the rest of the thallus develops entirely from the germinal disc. This disc is very conspicuous, though in certain views, when mounted and observed under the microscope, it is sometimes flattened out so as to suggest a flat plate growing in the same direction as that of the original tube (fig. 26), instead of being placed at right angles on the end of the latter. I have seen cases in which the direction of growth of the plate seemed really to be a continuation of that of the germ tube, but in all but one of these (fig. 24, Pl. XII) the sporeling had become separated from the spore wall, and hence could not be distinguished with certainty from the not infrequent cases of regeneration from small shreds of tissue in the soil. Regenerative shoots take on many different appearances, and often seem to grow for a time by means of a single two-sided apical cell, which is regularly wedge-shaped. The only resemblance to this condition that I have found in sporelings is in those grown in nutrient solution; in these instances the terminal cell seemed to function as an apical cell, forming a narrow ribbon composed of pairs of cells (figs. 35, 36, Pl. X), the whole gradually broadening out into a flat plate. In the latter stage, however, the appearance of an apical cell was lost, and lateral growing points often appeared, from each of which presumably, under favorable conditions, a thallus would have developed (fig. 37, Pl. XII; text fig. 1).

After the terminal disc has been formed, its further growth usually becomes markedly one-sided. This results from the development of a group of rapidly dividing initial cells at one side of the disc. This tendency is illustrated in figures 15, 17, etc., Plate IX. Division in this group of cells takes place in several planes all at right angles to the original divisions of the germ tube, so that a flat plate, one cell thick, grows out at right angles to the latter. At first the plate is cup-shaped, owing presumably to the more rapid growth of the edges; later, however, it spreads out into a flat blade. This process is illustrated in figures 17–26, Plates IX and XII. It is noteworthy that one of Campbell's figures (3) of a sporeling of Riccia bears a strong resemblance to some of these figures, such as figure 23. In most cases, growth occurs by the division of a few cells at one point on the margin of the plate. Sometimes two such groups of initials may be formed, resulting in a marginal growth of the thallus at two points (*a*, *a′*, figs. 27, Pl. XII, and 28, Pl. X). In the particular case shown in figure 27 there is



TEXT FIG. 1. An abnormal thallus which resulted from a spore germinated in nutrient solution: *a*, *a′*, *a″*, apical regions; *b*, basal cell; *r*, rhizoids. The cell walls at *a′* could not be seen on account of the density of the cell contents. Drawn from living material, × about 50. Compare figures 35 and 36, Plate X, and figure 37, Plate XII.

a cell at the apex of each lobe that has very much the appearance of an apical cell; but this is the exception rather than the rule. The typical group of initial cells is shown in figures 26 and 30–34 (Pls. IX, X). It consists of a marginal row of small cells of dense contents, rather longer than wide, all substantially alike as to size and shape. As these cells continue to divide, not enlarging to any extent, the segments cut off from them on the outside outstrip them in growth and form two lobes, one on each side of the apical region. This results in the formation of an apical notch, which may be median (figs. 26, 32, 33) or lateral (fig. 34). The lateral position of the apical notch is a common appearance; it is noted in the subsequent history of the plant in the formation of a small and a large lobe on opposite sides of the growing point, and frequently in the formation of short branches when the growing point forks.

After the young thallus has reached this stage in its development, it becomes more than one cell thick in the central portion. Just how this transformation is effected I have been unable to determine. Mounts *in toto* no longer prove satisfactory, owing to the density of the cell contents in the apical region; and the plants are still too small to be handled readily by ordinary methods of fixing and sectioning.

Apical Growth and the Formation of the Thallus Lobes

Both Campbell (2) and Leitgeb (9) describe the growth of the thallus as being due to a single apical cell which cuts off right and left lateral segments and dorsal and ventral segments. Campbell says that the lateral segments so resemble the apical cell in horizontal view that it is difficult to say with certainty that there is but one apical cell. Leitgeb describes the apical cell as lying at the deepest point of the notch in the forward margin of the thallus, and states that this notch is often so narrow that there is space only for a single small cell. He goes on to say, however, that sometimes the notch is broader, and more rarely it is quite wide, which appearance, he thinks, is a sign that forking is about to occur. The situation throughout, according to Leitgeb, is practically the same as that found in typical Ricciaceae and Marchantiaceae.

It is a difficult matter, in mature plants, to obtain sections passing through the growing point exactly in a longitudinal vertical direction, owing to the small size of the plant, and to the presence of a mass of involucres and lobes about the growing point. In many hundreds of slides, I have obtained two satisfactory series of sections of the growing point cut in this plane (figs. 38–54, Pls. X, XI), and some rather less satisfactory series cut in horizontal and in transverse vertical planes (figs. 55–67).

The plants which I studied were grown in a Wardian case in the greenhouse, and the shady conditions, the abundance of moisture, and the absence of any seasonal limitations of the growing period, were responsible for a more luxuriant growth of the thallus, with a corresponding increase in the size and number of the lobes, than is the case under natural conditions. The thallus, instead of being a simple small plate, the edges of which are cut into more or less crowded and overlapping lobes, as is the case in nature, took on the form of a central thick, distinct axis bearing leaf-like lobes irregularly on either side, and attaining a considerable size. The involucres also grew longer and were more cylindrical in form, the mouths often flaring out and becoming undulate. These peculiarities are even more noticeable if the plants are grown in petri dishes on filter paper moistened with a nutrient solution (fig. 70, Pl. XII). Campbell (2), working, according to Miss Haynes (8), on a mixture of *S. texanus* and *S. cristatus*, described similar forms occurring in nature under exceptional conditions of moisture and shade, but in these cases the production of sex organs was partly arrested, whereas in the plants I studied rather the reverse was true. The Douins (4, 6), studying *S. Michelii and S. texanus*, also mention the growth of abnormal plants under cultural conditions. According to the description of C. Douin (4), the plants usually possess in nature three large lobes with a small "middle lobe" in each of the two apical notches. The figures of Allen (1), however, made from living plants grown under natural conditions, show a less schematic and more luxuriant growth.

Leitgeb (9) says that the lateral segments of the apical cell continue for a time to cut off dorsal and ventral segments in the same manner as the apical cell itself, but sooner or later a lobe cell is formed, which grows out by vertical divisions into one of the lobes. This is the same condition as is shown by longitudinal vertical sections through the apical region (figs. 38–54). In such a case it is extremely difficult to determine whether there is a group of apical cells present, or a single apical cell, which has formed new apical cells to either side in preparation for a forking of the growing region. My figures show several small cells, having the shape usually described for the four-sided type of apical cell, grouped together in the apical region (*a*, figs. 38–54); and, in the case of one of the apical regions illustrated, several such groups are present, each two consecutive groups separated by a young lobe (*l*, figs. 38–48). The presence of several groups of initial cells indicates presumably that the original group had divided several times in close succession, the intervals between the groups being occupied by lobe cells. This is usually the case under cultural conditions, and dichotomy is correspondingly rapid. An examination of the plants under a hand lens confirms this interpretation of the figures. The apical region is often very wide, and there are usually from one to four small lobes in it. The fact that each group of initial cells consists of cells all alike and all having the appearance of four-sided apical cells, seems rather to favor the idea that growth is not due to a single apical cell but to a group of initials. It may be, of course, that the condition depends upon the environment, and that in nature a single distinct apical cell exists, while in culture this single cell is multiplied without a corresponding forking of the growing region necessarily resulting.

The existence of a group of initial cells is similar to the condition in Marchantia as usually described. Mottier (11), however, inclines to the view that in Marchantia also only one apical cell is present.

In longitudinal vertical section these initial cells (*a*, figs. 38–54) are regularly wedge-shaped, cutting off narrow segments mostly, but not always, in alternate succession. Archegonial initials may be seen, as described by Leitgeb (9), Douin (5), and others, only one or two cells distant from the initial cell or cells, each apparently formed from an entire dorsal segment of the latter (*ar*, figs. 38–54). From the ventral segments mucilage hairs grow out, each consisting of a row of cells, the terminal cell being large, spherical, filled with dense contents, and provided with a large nucleus (*s*, figs. 38–54). In the preparations these mucilage hairs become more or less shrunken and torn.

Sections cut through the growing region in a horizontal and in a vertical transverse plane are less satisfactory than those just described, owing to the even greater difficulty of orienting the plants in the paraffin for this purpose. Figures 55–61, Plate XI, illustrate a series of horizontal sections, starting at the ventral side. The small cells marked *a* in figures 59 and 60 probably represent the apical group. They are noticeably more dense in content than any other cells in the sections, except those of the mucilage hairs. They are obviously dividing with considerably rapidity; and the varying length of the same cells in succeeding sections (*x*, figs. 59, 60) makes it probable that they are bounded posteriorly by an inclined wall, and therefore correspond to the initial cells shown in the vertical sections. The sections suffer from the fact that they are cut at a slight angle from the horizontal.

Figures 62–67, Plates X and XI, illustrate similar groups of cells as seen in vertical transverse section. The first series (figs. 62–64) is taken from a very young thallus, consisting of a simple plate one cell thick in all parts save in the center, where the cells are smaller, denser, and obviously embryonic. At the beginning of the series (fig. 62) there are two plates of cells, one cell thick, separated by an empty space—the apical notch—instead of by the group of initials. At one side in the drawings (*x*, figs. 63, 64) there are several large hyaline cells which disturb the otherwise symmetrical arrangement. This is probably due to a fold in the thin thallus-blade. Figures 65–67, Plate X, illustrate a similar series from a mature plant. Here also a group of similar embryonic cells (*a*) is present; but in this case they are cut slightly on a bias, so that at one side they seem to merge into a mature lobe (*l*), while at the other side there is no corresponding structure. It is unfortunate that none of the sections except those cut in the longitudinal vertical plane showed the young lobes so evident in the latter; unless the cells marked *y*, figures 57–60 (Pl. XI), may be interpreted as such.

A second method was used in order to determine the origin of the lateral lobes of the thallus. Single plants were isolated and grown in separate pots, and sketched each day under a binocular microscope. Young plants with only two or three lobes were selected in most cases, and watched in this way until they had attained the size of normal mature plants. The lobes differ sufficiently in shape and size so that one can feel certain that one is following the growth of each individual lobe from its beginning until maturity. The plants were kept in the Wardian case and showed the peculiarities of growth already described.

This study shows that the lobes of the thallus are formed at the tip and are pushed back into a lateral position, as they increase in size, by the elongation of the median portion of the thallus, or midrib. It is also seen that they are not "middle lobes" in the ordinary sense, that is, lobes produced simply by the outgrowth of the thallus between two divisions of the originally single growing region; for very commonly two lobes are formed at the same time, without any consequent branching of the thallus. If all the notches between the lobes represent divisions of the apical region, it must be that some of these branch regions are arrested in further development, and that their cells merely mature without dividing further. When one lobe is formed singly, it often resembles the ordinary "middle lobe" of such a form as *Ricciocarpus natans*, and the growth of the thallus continues on either side of the lobe. This, however, is not necessarily the case, since in many cases only one of the regions to either side of the lobe produces new growth, the middle lobe being pushed aside and coming to lie on one side of the central axis, without a corresponding lobe on the other side. This is of common occurrence, and is often responsible for the curving growth of the thallus as a whole.

In the light of the foregoing studies, therefore, it is reasonable to suppose that a lobe is formed by the occurrence of vertical divisions in a lateral segment of an initial cell, and this, as both the sections and the study of living plants show, may, at least in greenhouse cultures, occur anywhere in the apical region, irrespective of divisions of the latter.

In nature, the growth of the thallus, to judge from the accounts of previous authors, is apparently much like that of Riccia. According to Douin's (5) description of Sphaerocarpos, there are usually two notches, separated by a large middle lobe, present in a mature plant. In the notches are usually two other small lobes, with a growing point on either side—thus four growing points in all. At this point, he says, growth usually stops, though it is not uncommon to find plants having five large lobes with small ones in each notch. The growth, of course, is limited by the end of the growing season. The only difference between this history and that of Riccia, according to the same author, is the fact that in Sphaerocarpos the middle lobe remains undivided, whereas in Riccia it becomes cleft as the midrib elongates. Hence in the latter case the growth of the midrib is more rapid than the intercalary growth at the base of the lobes, while in the former case the reverse is true. In nature, evidently, the lateral segments of initial cells of the apical region merge together in their development to form the broad marginal wing of the thallus; while in culture the more rapid growth of the central portion of the thallus causes the separation of the tissue developed from each cell into separate lobes.

In the plants grown in culture, the involucres are often broadly open at the tip and show various irregularities in form. Dorsal lobes were observed in several cases, and there are gradations between these dorsal lobes and the normal involucres. The Douins (6) also mention these peculiarities. This suggests the idea that the formation of lobes and that of involucres may be intimately connected, and that the form of the entire plant may depend rather strictly upon the environment. One might go even farther and suggest that there is an evolutionary relationship between some of the higher lobed or leafy liverworts and the various forms assumed by the more primitive types. There is at least a close resemblance. In *Pellia epiphylla*, according to Douin (4), of two middle lobes produced by the forking of the growing point, one is arrested in growth, while the other continues its development, and then a new lobe appears in the notch between them, thrusting to one side the lobe which has ceased its growth. The lateral lobes of many liverworts may be formed in this way.

Plants grown under water by regeneration from involucres and from lobes cut off from the plant show the same abnormalities as those above described carried to an extreme, except that there is little or no branching and that sex organs are not abundant. The plant consists of a long almost cylindrical axis, bearing a few scattered and small leaf-like lobes.

The following is a detailed account of two of the plants studied as indicated above. They are typical of the history of all the other plants studied, so that it is needless to multiply examples.

Plant no. 1. Lobes *a*, *b*, *c*, *e*, and *f* (fig. 68 *A*, Pl. XII) have already reached maturity, and do not change their position in the subsequent history of the plant; they may increase slightly in size. Lobe *d* is an example of a "middle lobe," growing regions developing on either side of it. However, in the notch to the left of it, a single lobe forms (*g*, fig. 68 *B*), on only one side of which—between lobes *g* and *d*—is there any further growth. In the notch between these two lobes growth proceeds in the usual way. On the other side of *d*, at first only one lobe—*h*—is visible (fig. 68 *B*), but another—*i*—soon makes its appearance (fig. 68 *C*), and finally comes to equal *h* in size. This sort of thing is of common occurrence, and indeed the formation of these little lobes exhibits the greatest variability as to size, shape, number, and time of appearance. The further history of this plant illustrates how the lobes are pushed into a lateral position, while new lobes arise at the apical region. In the last sketch (fig. 68 *D*), lobes *h* and *i* are approaching their mature size, and two small lobes have made their appearance simultaneously between them. On the other side of lobe *d*, meanwhile, two lobes (*k* and *j*) have developed at the same time (fig. 68 *C*), and finally (fig. 68 *D*) are considerably spread apart and a single lobe is to be seen between them.

Plant no. 4. The two forward lobes, *a* and *b* (fig. 69 *A*), are spread apart until there is a wide notch between them and they occupy a lateral position (fig. 69 *B*). This notch is filled by a round mass of cells. Two new lobes, *c* and *d* (fig. 69 *C*), are formed simultaneously in this notch, at first being in a transverse and nearly vertical position. (In the drawing, these two lobes appear to be of dorsal origin. This is not actually the case, the illusion being due merely to the position in which the plant was seen when sketched.) These two lobes are then subjected to the same process of spreading, and a few days after (fig. 69 *D*) a new lobe, *e*, has appeared between them. Three days after this, this lobe has reached a considerable size, and there is a new small lobe on either side of it—lobes *f* and *g* (fig. 69 *E*). This occurrence of three lobes, not widely different in size, is also very common. In the last stage sketched (fig. 69 *F*), a single lobe—*h*—again appears on one side of this "middle lobe," and on the other side two small lobes, *i* and *j*, are formed at the same time. In the subsequent history of this plant, the formation of lobes went on in the same way, small lobes succeeding each other rapidly in a very various and complicated manner, with no apparent relation to the occurrence of branching except sometimes in cases where only a single lobe was formed at one time (*e.g.*, lobe *e*, fig. 69 *F*).

Branching always occurs by the division of the apical region. In nature it is limited by the short growing season of the plant, and the number of growing points found in plants living under natural conditions, as described by Douin, has been referred to above. In culture, however, the plants may live indefinitely, and profusion of branching is in keeping with the luxuriant habit of the plant as a whole under greenhouse conditions; and owing to the rapid elongation of the central axes of the branches, the latter are more easily distinguished from each other. By the gradual dying of the posterior part of the thallus, branching, under the conditions referred to, is an effective means of vegetative multiplication.

SUMMARY

1. The spore of *Sphaerocarpos Donnellii* germinates by means of a slender filament of cells, the germ tube, on the end of which, and at right angles to it, is formed a germinal disc. The latter structure develops into the thallus of the mature plant.
2. The history of the formation of the germ tube is very variable.
3. The growth of the germinal disc does not seem to be due to the activity of a single apical cell, except in special cases. It is formed by divisions of all the terminal cells of the germ tube, and continues its growth through the activity of a group of cells on the margin which remain embryonic in character.

4. The apical growth of the mature thallus is due to a group of cells occupying the apical notch. These cells have the appearance of four-sided apical cells. The lateral segments which they cut off either resemble the mother cell, or go to the formation of the thallus lobes. The dorsal and ventral segments add to the thickness of the thallus in the median portion.

5. The marginal lobes of the thallus are formed by the division of lateral segments of the apical cells. Under natural conditions, successive lateral segments merge together in their development to produce a more or less continuous marginal wing. Under cultural conditions, the more rapid elongation of the median portion of the thallus causes the separation of the structures derived from individual lateral segments into distinct leaf-like lobes, attached laterally along a central midrib.

6. Branching of the thallus is due to a division of the apical group of cells into two such groups, a lobe occupying the region between. The formation of lobes is not necessarily related to branching.

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EXPLANATION OF PLATES IX–XII

All drawings, except figures 68, 69, and 70, were made with the aid of a camera lucida. In these cases the drawings were freehand sketches of plants as seen under a binocular microscope. Figures 25, 35, 36, 37, 68, 69, and 70 were made from living plants. Magnifications given are approximate.

Fig. 1. Undivided germ tube. × 150.

Fig. 2. First division of the germ tube: *sp*, spore wall. × 150.

Fig. 3. Second division of the germ tube. × 150.

Fig. 4. Third division of the germ tube: *sp*, spore wall; *r*, rhizoid. × 150.

Figures 1–4 are shaded to indicate the distribution of the dense cell contents.

Figs. 5–9. Further divisions of the germ tube, prior to the formation of the germinal disc: *sp*, spore wall; *b*, basal cell. × 150.

Fig. 10. Beginning of the formation of the germinal disc: *a*, possible apical cell. × 350.

FIG. 11. Further divisions in the formation of the germinal disc: *b*, basal cell. × 150.

FIG. 12. The same: *a*, possible apical cell; *b*, basal cell. × 350.

FIG. 13. Two sporelings at the time of formation of the germinal disc: *sp t*, spore tetrad; *r*, rhizoid; *g*, germinal disc. × 50.

FIG. 14. Formation of the germinal disc (compare fig. 13, *g*). × 350.

FIGS. 15–23. The young germinal disc. × 150.

FIG. 24. Young thallus continuing its growth in one plane, without formation of a germinal disc: *a*, possible apical cell; *sp*, spore wall. × 75.

FIG. 25. Germinal disc in a more advanced stage: *a*, apical region (the cell contents too dense to permit of the cell walls being distinguished); *b*, basal cell; *c*, place of attachment of the germinal disc to the germ tube. × 75.

FIG. 26. The initial cells at the apex of a young sporeling. × 150.

FIG. 27. Young thallus growing out in two directions, and possessing two apical regions: *a*, *a'*, apical regions; *c*, place of attachment of germinal disc to germ tube; *b*, basal cell. × 75.

FIG. 28. The apical regions of the plant shown in figure 27: *a*, *a'*, possible apical cells. × 150.

FIG. 29. The point of attachment of germinal disc to germ tube. × 150.

FIG. 30. Initial cells of a young sporeling. × 150.

FIG. 31. The same. × 350.

FIGS. 32, 33. The same in slightly older sporelings, showing the formation of the apical notch. × 150.

FIG. 34. The formation of the apical notch in a lateral position. × 150.

FIG. 35. Abnormal sporeling from a spore germinated in nutrient solution. × 25.

FIG. 36. Detail of figure 35. × 150.

FIG. 37. Another abnormal thallus produced in the same way: *a*, apical region; *r*, rhizoid. × 75.

FIGS. 38–48. Series of longitudinal vertical sections through the apical region of a mature plant: *a*, apical cells; *ar*, archegonia; *i*, involucres; *l*, lobes of the thallus; *s*, mucilage hairs. × 350.

FIGS. 49–54. Another series of sections through the apical region of a mature plant, cut in the same direction as the preceding: *a*, apical cells; *ar*, archegonia; *ar in*, archegonial initials; *i*, involucres; *l*, lobes of the thallus; *s*, mucilage hairs. × 350.

FIGS. 55–61. Series of horizontal sections through the apical region of a mature plant. Two sections omitted between figures 60 and 61. The first figure of the series is on the ventral surface of the apical region, the last one on the dorsal surface: *a*, apical cells; *ar*, archegonium; *ar in*, archegonial initial; *l*, lobe of the thallus; *s*, mucilage hairs; *x*, see text; *y*, possible lobe. × 400.

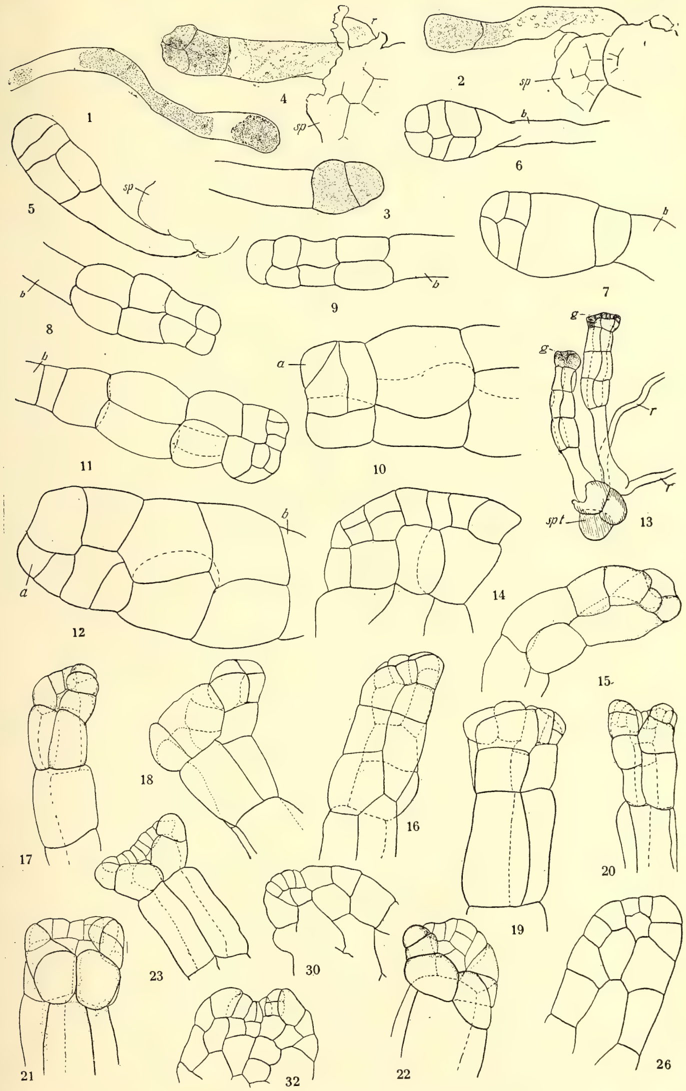
FIGS. 62–64. Series of vertical transverse sections through the apical region of a young plant: *a*, apical cells; *s*, mucilage hair; *X*, see text. × 400.

FIGS. 65–67. Series of sections through the apical region of a mature plant cut in the same way as the preceding series, but in a plane not quite perpendicular to the axis of growth: *a*, apical cells; *ar*, archegonia; *i*, involucres; *l*, lobe of the thallus; *s*, mucilage hairs. × 350.

FIGS. 68, 69. Two plants sketched at intervals of a few days in order to follow the origin and growth of the lobes of the thallus. Corresponding letters indicate corresponding lobes in each series. The following table shows the time which intervened between successive sketches.

FIG. 68*A*......	March 8.	FIG. 69*A*........	March 8.
FIG. 68*B*......	" 13.	FIG. 69*B*........	" 12.
FIG. 68*C*......	" 16.	FIG. 69*C*........	" 14.
FIG. 68*D*......	" 19.	FIG. 69*D*........	" 19.
		FIG. 69*E*........	" 22.
		FIG. 69*F*........	" 28.

FIG. 70. Plant grown under conditions of excessive moisture, in a petri dish.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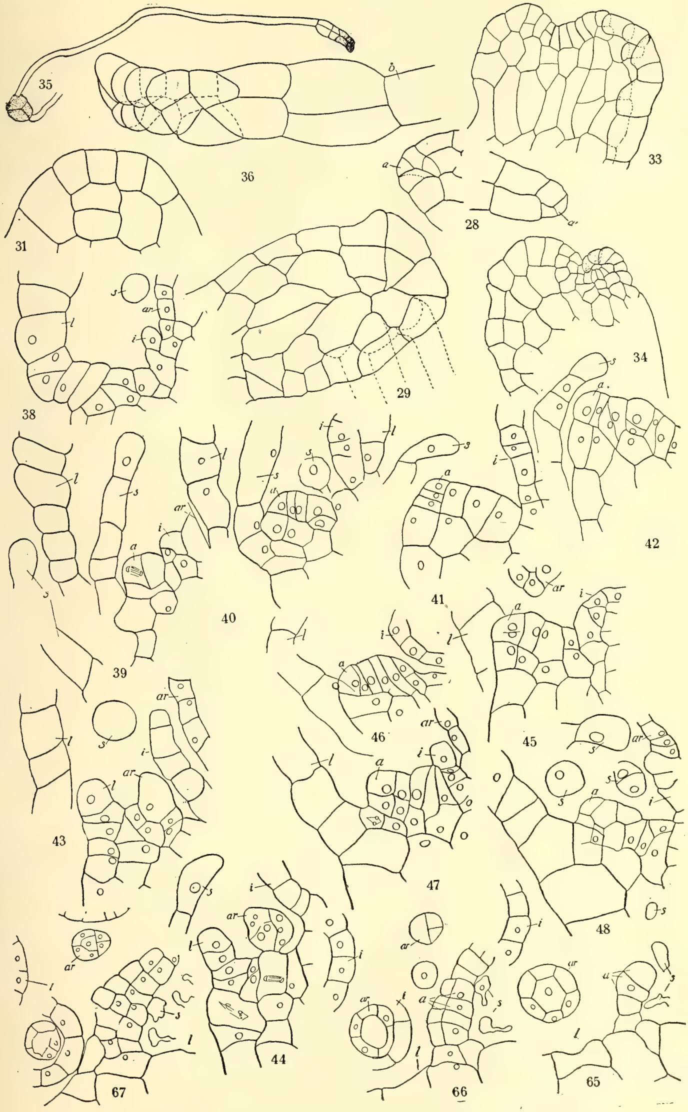
RICKETT: DEVELOPMENT OF SPHAEROCARPOS DONNELLII.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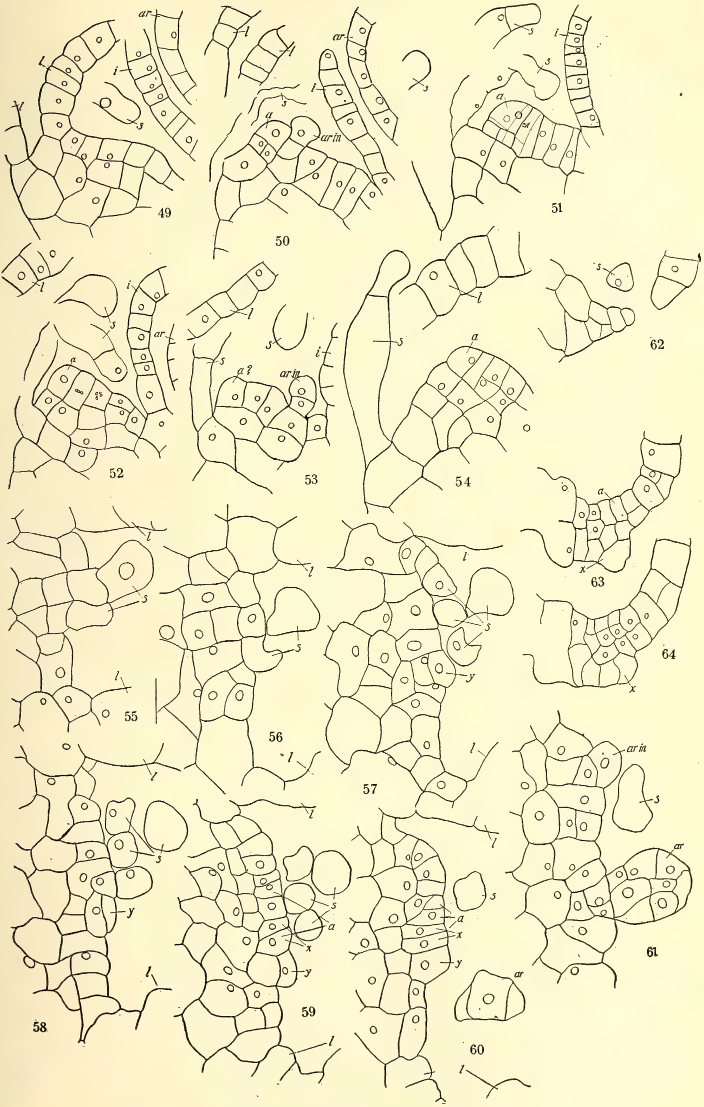
RICKETT: DEVELOPMENT OF SPHAEROCARPOS DONNELLII.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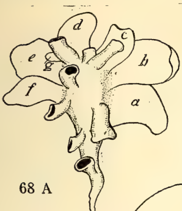
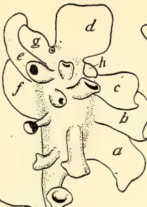
RICKETT: DEVELOPMENT OF SPHAEROCARPOS DONNELLII.



68 A



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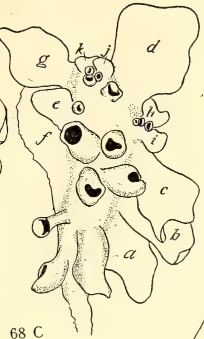
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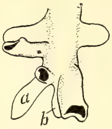
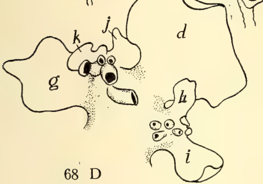
68 C



69 A



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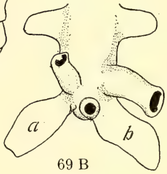

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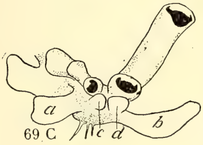

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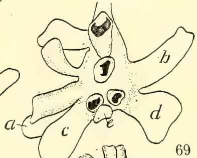

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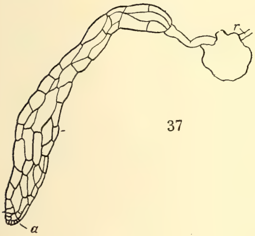

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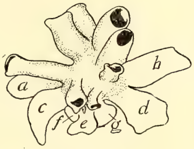

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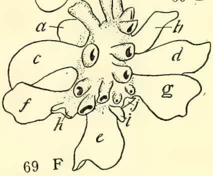
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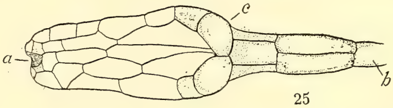
69 F



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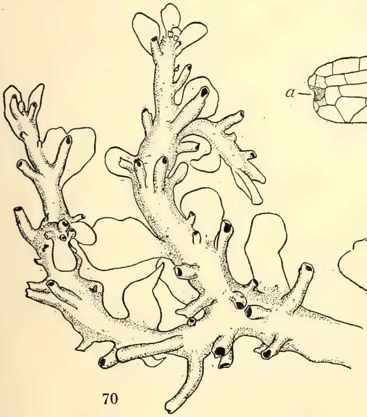

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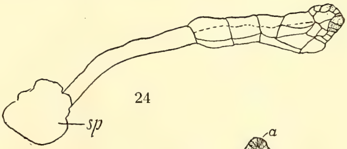

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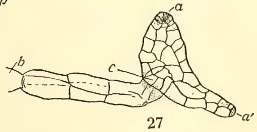

27

RICKETT: DEVELOPMENT OF SPHAEROCARPOS DONNELLII.

THE GENUS PLANTAGO IN HAWAII

Joseph F. Rock

The Hawaiian Islands possess two endemic species of Plantago, of which one, *Pl. princeps*, is a branching shrub. The only other known species which is a branching shrub is *Plantago fernandezia*, a native of the island of Juan Fernandez. As far as we know *Pl. fernandezia* is not a variable species, while *Pl. princeps* is exceedingly variable and is represented in Hawaii by eight varieties. Curiously enough the typical form has not been collected since the days of Hillebrand.

Plantago princeps is primarily a plant of the lower and drier region, occurring only on the leeward side of Oahu, Kauai, and Molokai. It is true some forms grow near waterfalls (var. *longibracteata*) and exposed to the force of the water, and others grow on drier slopes in stands (var. *elata*). Three varieties of *Pl. princeps* grow usually near water courses (var. *denticulata*, var. *longibracteata*, and perhaps var. *hirtella*). The stemless form (var. *acaulis*) occurs in the rain forest on clay banks, as does var. *Queleniana*. *Plantago princeps* var. *elata* reaches a height of six feet, while var. *denticulata* with a height of four feet is the next tallest; all forms occur from these heights to short, simple stems and stemless plants.

Wawra, who studied the different forms of this and the other species more thoroughly than any previous botanist, lays especial stress, and that rightly, on the venation, which is parallel and free in *Plantago pachyphylla*, while in *Plantago princeps* the lateral veins converge and join the median nerve. The pyxidium dehisces at the base in *Pl. princeps;* in *Pl. pachyphylla* it dehisces at the middle. The seed in *Pl. princeps* is viscous, linear, and black, while in *Pl. pachyphylla* the seeds are supposedly oval, light brown, and not viscous. None of the characters, including the branching and stemless habit of the two species, hold good, nor can they be relied upon as specific characters. This brings us to the conclusion that both species are closely related and even hybridized, which is proven by the numerous forms exhibited by both species. Yet if one should take the very small forms of *Pl. pachyphylla* (var. *pusilla*) from the summit swamp of Kauai, Mt. Waialeale, and compare them with the var. *mauiensis*, one could describe them as distinct species, although there are gradations to be found which to a large extent link these two varieties together.

The main link between *Pl. princeps* and *Pl. pachyphylla* is furnished by a new variety (var. *anomala*) of the former species. That variety has the capsules and leaf venation of *Pl. pachyphylla*, but the seeds and arborescent branching habit of *Pl. princeps*. Variety *acaulis* of this latter species is also intermediate between the two species and evidently comes closest to *Pl. pachyphylla* var. *hawaiiensis.* It has the capsule and seeds of *Pl. princeps*, but is stemless and has the habit of var. *hawaiiensis* of *Pl. pachyphylla.*

Characters such as pubescence of spike and leaves are not reliable, as glabrous and pubescent spikes occur on one and the same plant. The wool which often covers the under side of the leaves is permanent, while on the spike it is deciduous. Pubescence or slight hairiness disappears often entirely in older plants, while it is present in younger, flowering specimens. The stem in *Plantago princeps* is hairy in var. *Queleniana*, but glabrous between the nodes in other varieties and often even glabrous on the leaf scars. The stamens and style are long exserted in all forms of both species with the exception of var. *Queleniana* according to Gaudichaud's drawing, and also in the typical form as described by Chamisso and Schlechtendal. Male spikes, however, have not been found and that statement cannot be verified at present. As regards the characters of the seeds in the two species, the difference is very slight; the seeds of *Pl. pachyphylla* are not greatly different from those of *Pl. princeps*, and the number of seeds in each locule is also variable. In *Pl. pachyphylla* the seeds are recorded as oval and light brown. The seeds examined of that species by the writer are all dark brown and oblong rather than oval; the margins of the seeds are lighter because of their being transparent.

A decidedly interesting variety of *Pl. pachyphylla* was discovered by the writer on the high, swampy plateau of the Kohala mountains. While the wool in all the varieties of *Pl. pachyphylla* is light brown or fawn-colored, the Kohala variety is densely covered with long, stiff gray hairs. The leaves are very thick and brittle and, including the length of the hairs on both surfaces, are fully 2.5 cm. or an inch thick. The numerous spikes are also densely hairy. This variety grows in sphagnum in open bogs. The capsule and seeds are those of *Pl. pachyphylla;* otherwise the writer would describe it as a new species because of the very different vegetative characters.

One would come to the conclusion that location has a good deal to do with the plant habit; this is not the case with these species, although it may have some influence. The main reason, to the writer's mind, is hybridization, for we find three or four varieties growing together side by side, as is the case with var. *pusilla*, var. *glabrifolia*, var. *rotundifolia*, and var. *kauaiensis* of *Pl. pachyphylla.* So far the first variety has been found only on Kauai, while var. *rotundifolia* occurs also on Maui on the summit swamp of Mt. Eeke, in a modified form (oblong leaves instead of suborbicular). Variety *kauaiensis* is represented by a form (forma *robusta)* both on Maui and Molokai; on the latter island the plant is less robust than on Maui. Variety *glabrifolia* seems to be a large, glabrous form of var. *kauaiensis* and comes very close to large, glabrous forms of forma *robusta* from Molokai. While these intermediates occur, numerous individuals of each apparent variety grow side by side.

Plantago pachyphylla is mainly a bog plant, though the typical form, var. *α mauiensis*, grows in the drier regions in the uplands of Haleakala, Maui, along dry stream beds in company with *Geranium tridens*, *Argyroxiphium sandwicense*, *A. virescens*, *Raillardia platyphylla*, *Styphelia Grayana*, etc.

The only other variety, if not a distinct species (var. *hawaiiensis*), grows in the dry cinder on the upper slopes of Mt. Hualalai at 6,000 to 7,000 feet elevation, and also on Mauna Loa, on the island of Hawaii. All the remaining varieties are bog plants or occur immediately below the bogs in moss forests, especially in open places on the ridges. In the bogs they are associated with Viola, *Acaena exigua*, *Geranium humile*, and certain Compositae, as Dubautia and Wilkesia, and on Kauai also with Drosera. Other plants found in its company are *Carex montis eeka*, *Eragrostis variabilis* (on Maui), Scaevola (also on Maui), and Lobelia, as well as Trematolobelia and, on Maui, Argyroxiphium.

Plantago pachyphylla is said to be closely related to *Plantago aucklandica* from the Auckland Islands, while *Pl. princeps* is closely related to *Pl. fernandezia* of Juan Fernandez. The writer has not seen specimens of these two species and is not able to say whether these contentions are correct.

The two Hawaiian species occur on all the islands of the group with the exception of Lanai, Niihau, and Kahoolawe; this does not mean, however, that they never occurred there. In all probability *Pl. pachyphylla* was absent from the two latter islands owing to their dryness and low altitude while it may have occurred on Lanai and there is a possibility that specimens may yet be found there, although that is doubtful, the island being much drier now than it ever was previously.

In working up these difficult and perplexing forms of *Pl. princeps* and *Pl. pachyphylla* the writer had at his disposal the collection in the Gray Herbarium and duplicates of Hillebrand's collection which were given him by the authorities of the Berlin Herbarium.

The writer is indebted to Mr. E. H. Bryan, Jr., a student of the College of Hawaii, for securing references and original descriptions from books not in the College of Hawaii library and for copying the manuscript. He is indebted to Dr. B. L. Robinson of the Gray Herbarium for the loan of the Hawaiian Plantago material in the Gray Herbarium, and he takes this opportunity to express his sincere thanks.

KEY TO THE HAWAIIAN SPECIES AND VARIETIES OF PLANTAGO

A. Lateral veins converging with the median nerve; pyxidium circumscissile at the base (excepting *Pl. princeps* var. *anomala*). *Plantago princeps.*
  B. Stems woody, branching or simple and erect.
    C. Stems simple and erect, not branching.
      D. Stems hairy or woolly their whole length, leaves broadly stem-clasping. var. *Queleniana.*
      D′. Stems decidedly glabrous, leaves contracted at the base but broader at the insertion. var. *elata.*
    C′. Stems branching.

D. Leaves petiolate.
E. Leaves glabrous, loosely arranged. var. *laxifolia.*
E′. Leaves hirtellous or hispid on both surfaces.
F. Petioles slender, distinct. var. *hirtella.*
F′. Petioles broadly winged, indistinct and broadly dilating at the base. var. *denticulata.*
D′. Leaves broadly sessile, linear-lanceolate, 30 cm. long by 4 cm. broad, veins parallel; pyxidium dehiscing at the middle. var. *anomala.*
B′. Plants stemless or very short-stemmed, herbaceous and drooping.
C. Leaves pubescent above with scattered hairs; floral bracts long, acuminate. var. *longibracteata.*
C′. Leaves glabrous and darker above, pale beneath, with dark-brown pubescence on the prominent nerves below. var. *acaulis.*
A′. Veins free, parallel or arcuate, not converging; caudex simple, never branching; pyxidium circumscissile at the middle. *Plantago pachyphylla.*
B. Leaves glabrous on both surfaces or slightly pubescent beneath.
C. Rosettes large; leaves large, 15 to 18 cm. long by 5.5 to 10 cm. broad, glabrous on both sides, 9- to 11-nerved. var. *glabrifolia.*
C′. Rosettes small; leaves 5 to 6 cm. long, 3- to 5-nerved, often slightly pubescent beneath. var. *kauaiensis.*
B′. Leaves pubescent, puberulous, or woolly on one or both surfaces.
C. Pubescence or woolliness fawn-colored.
D. Leaves pubescent or woolly beneath.
E. Leaves pubescent beneath, sessile; spike woolly and stout. var. *mauiensis.*
E′. Leaves orbicular in outline, densely matted below with fawn-colored wool. var. *rotundifolia.*
D′. Leaves glabrous beneath, strigosely hispid above; plants small, rosette-like; spike few-flowered. var. *pusilla.*
C′. Pubescence gray.
D. Leaves puberulous, linear-lanceolate; spikes glabrous, slender. var. *hawaiiensis.*
D′. Leaves thick, brittle, broadly ovate, densely hirsute with gray hairs on both sides; spike hirsute. var. *muscicola.*

PLANTAGO PRINCEPS Cham. Schlecht. Linnaea **1**: 167. 1826.

A shrub 1.3 m. high, branching; branches flexible, terete, naked; leaves deciduous, leaving scars, dilating at the base, semi-amplexicaul, very glabrous, usually 15 cm. long, 2.5 cm. broad, linear-lanceolate, acuminate, narrowed before dilating at the base, 7- to 9-nerved, sometimes oval-lanceolate, shorter, 5 to 7.5 cm. long, 16 to 20 mm. broad, less acute at the apex; margin either entire or denticulate with few minute teeth especially in the upper half; axils woolly, the wool ferrugineous; spike pedunculate, exceeding the leaves, elongate, lax, sparsely flowered, attenuate at the apex; peduncles shorter than the leaves, axillary, compressed, glabrous or with few hairs; flowers lax; bracts glabrous, sometimes ciliate, shorter than the calyx, resembling the sepals but narrower, woolly at the base; calyx 4-sepalous; sepals ovate-acute, the back brown, the margins whitish, membranaceous, lightly erose, sometimes ciliate; corolla longer than the calyx, segments erect-spreading, lanceolate, acuminate; stamens inserted at the base of the corolla-tube, *never* exserted; filaments short; anthersoblong, hastate at the base, brown; style very long, three to four times the length of the corolla; stigma short; capsule scarcely longer than the calyx, ovate-elliptical, mucronate, two-seeded in each locule, circumscissile above the base; seeds black.

In the valleys of the lower mountain ranges of Oahu (O-Wahu).

OAHU: Kalihi Valley, Hillebrand in herb. Berlin and College of Hawaii Herb. no. 16004.

There are no specimens extant in the herbarium at Harvard of the typical *Pl. princeps* Cham. Schlecht. Chamisso states that the stamens are *never* exserted while Hillebrand says "long-exserted." All the varieties referable to this species have long-exserted stamens, with the exception of var. *Queleniana* (Gaud.) Rock which brings that variety closer to the species than any others.

The typical form has not been collected by the writer, nor was it found by Wawra or Heller. Wawra lays especial stress on the venation of the leaves, the veins converging with the median costa below the middle, while those of *Pl. pachyphylla* have the nerves free to the base and parallel to each other.

One exception occurs in *Pl. princeps* var. *anomala*, a new variety collected by Heller on Kauai; the leaves of that variety are those of forms of *Pl. pachyphylla* while the seeds are linear-oblong and black as in *Pl. princeps*. The stem of the new variety is four feet high and branches in a candelabra-like manner, while *Pl. pachyphylla* is stemless. The new variety is apparently intermediate, connecting the two species, or it can be looked upon as a new species; the writer prefers the former interpretation.

Chamisso also says "style glabrous"; the style in the specimen examined is hairy, as in all the varieties of the species. Wawra's statement that the capsules of variety *laxifolia* dehisce at the middle is incorrect.

The only constant character for *Pl. princeps* is the linear-oblong, black seeds. Nervature and branching habit are not constant characters, as stemless plants appear in *Pl. princeps* and the nerves of *Pl. princeps* var. *anomala* are not converging as in all other varieties; the dehiscing of the capsule is also not to be relied upon as a specific distinction, for an exception occurs in the last mentioned variety. From all this it may be seen that *Plantago princeps* is probably the older of the two species.

PLANTAGO PRINCEPS var. **Queleniana** (Gaud.) Rock.

Plantago Queleniana Gaud. Bot. Voy. Uranie 445, t. 50. 1826.

Stem woody, erect, simple, terete; foliose at apex; hairy or woolly all along the stem, especially toward the apex and at the leaf-scars; leaves thick, subcoriaceous, oblong-lanceolate to oval-lanceolate, glabrous on both sides, acute or sub-acuminate at the apex, gradually narrowing at the base, broadly sessile, partly stem-clasping, 5 to 12 cm. long, 11 to 30 mm. broad, 5- to 11-nerved; nerves converging near the base with the median costa; spikes 1 to 5, more than twice the length of the leaves, peduncles shorter than or equaling the leaves; flowers as in the species; stamens not exserted.

OAHU: Gaud. in herb. Museum Paris; Gaud., Voy. Bonite, in Gray Herb.; U. S. Explor. Exped. in Gray Herb.; J. Remy, no. 427 in Gray Herb. ex herb. Museum Paris; Seemann, no. 2263 in Gray Herb.; Mann & Brigham, no. 85 in Gray Herb.; Manoa Valley, Rock, 1915, College of Hawaii Herb. no. 16001.

Pl. princeps var. *Queleniana* differs from the typical species in the simple, erect stem which is woolly throughout, while that of the species is glabrous. The leaves are not petiolate, but are more or less broadly stem-clasping. The stamens are also included, while in all the other varieties they are exserted. Bennett's *Plantago Queleniana* in the Berlin Herbarium belongs to *Pl. pachyphylla*. Heller's "*Pl. Queleana*," no. 2610, and marked *Pl. princeps* in the Gray Herbarium has nothing in common with this variety; in fact, it represents a very anomalous form.

In Hillebrand's collection in the Gray Herbarium there is a specimen collected in the Kohala mountains from the north coast of Hawaii, which he refers to *Plantago princeps* var. *laxifolia*, but which comes close to var. *Queleniana;* however, owing to the glabrous and somewhat petiolate leaves it is here omitted and referred to var. *laxifolia.*

Pl. princeps var. *Queleniana* is decidedly a montane variety and restricted to the rain forests, while the other varieties including the species occur on the outskirts of the forest and in the drier localities in the lower valleys.

PLANTAGO PRINCEPS var. ELATA Wawra Flora **32**: 563. 1874.

Shrub 2 m. high; stem undivided, erect, terete, foliose at the apex, otherwise naked, decidedly glabrous; leaves glabrous, lanceolate, 12.5 cm. long, 2.5 cm. broad, acuminate, narrowing at the base, and about 8 mm. broad at the insertion on the stem, glabrous, shiny above, 9-nerved, the 2 or 4 inner nerves confluent below with the median nerve; spikes many (10 to 20), axillary, twice as long as the leaves; peduncle 7.5 to 10 cm. long, densely flowered, glabrous; bracts half the length of the calyx-lobes; flowers glabrous at the base; calyx lobes ovate-acute; tube of corolla equaling the calyx, the segments linear-lanceolate, acute, reflexed above the calyx; ovary ovate-obtuse; style filiform, long exserted; capsule oblong, twice as long as the calyx, long apiculate, circumscissile near the base, bilocular, locules one-seeded.

OAHU: Mountains of Waianae, Wawra, no. 1728 *b*, in herb. Vienna; specimen not seen.

This is not a synonym of *Queleniana* as cited by Drake del Castillo. It differs from *Queleniana* in the perfectly glabrous stem, which is even glabrous between the leaves. The stem is also unbranched. It is found in the dry regions of the Waianae mountains, while *Queleniana* is a montane species of the rain forest.

This variety differs from the species and from other varieties mainly in the simple, six-foot-tall stem which is glabrous, in the numerous spikes, and in the one-seeded locules. According to Wawra this variety has the appearance of a small palm and forms dense, almost impenetrable stands on the declivities of the lower Kaala range.

Plantago princeps var. laxifolia A. Gray, Proc. Amer. Acad. 6: 54. 1866.

Stem 30 to 60 cm. high, more or less woody, hollow, with deciduous wool or straight, silky, fawn-colored hairs in the axils of the leaves; leaves 10 to 15 cm. long, obovate-oblong, submembranous, 4 to 5 cm. broad, acute at the apex, gradually narrowing at the base into a winged petiole of 2.5 to 4 cm., 7- to 9-nerved, glabrous on both sides; spikes numerous, 30 to 45 cm. long, glabrous or with a few hairs at the base of the corolla, densely flowered; corolla-tube one third longer than calyx, segments linear, reflexed; capsules slightly longer than the calyx, obtuse, 4-seeded.

Hawaii: Stones by seaside, north base of Mauna Kea, U. S. Explor. Exped., in Gray Herb., specimen seen.

Maui: Ravines back of Lahana, Hillebrand ex herb. Berlin, in College of Hawaii Herb. no. 16,002.

Kauai: Waialeale (about 5,000 feet), no. 2204 in herb. Vienna, Wawra. (Wawra's description agrees well with the type in Gray Herb.)

Variety *laxifolia* which is marked "*laxiflora*" in Gray's handwriting on the type specimen, differs apparently very little from *Pl. princeps* var. *denticulata* Hillebr., the only difference being the hirsute or pubescent leaves and spikes. The denticulation of the leaves in var. *denticulata* is really not a distinguishing character as it occurs in other varieties. Wawra's statement that var. *laxifolia* is the only variety whose stem is glabrous is wrong, as glabrous stems occur also in var. *denticulata*.

Though Wawra's description of his species which he refers to Gray's variety *laxifolia* agrees with the latter's type, with the exception that there are no cilia present in the type on the margins of the sepals, it is hardly believable that plants of such widely differing localities as the beach of the north coast of Hawaii and the summit of Waialeale could be the same. The writer has not seen Wawra's specimens in the Vienna Herbarium and consequently cannot settle the question. There is a possibility that Wawra wrongly recorded the locality. He speaks of having two plants from Waialeale referable to this variety, one a very slender specimen, sparsely flowered and with long petiolate leaves and acute sepals, while the other, a more mature specimen, has a thicker stem, stiffer and shorter petiolate leaves and obtuse sepals. The fact that he says that the latter specimen may come close to a plant described by Gray as *Plantago pachyphylla* var. *hawaiiensis* subvar. *gracilis* leads us to suspect that he actually had a plant of the *pachyphylla* type rather than one belonging to *princeps*, especially as *Plantago pachyphylla* is represented on Waialeale by numerous varieties.

Plantago princeps var. hirtella A. Gray, Proc. Amer. Acad. **6**: 54. 1866.

Stem erect, 60 cm. high, hollow, with permanent wool in the axils or straight, silky hair of a rich brown color; leaves oblong-elliptical, flaccid, 10 to 14 cm. long, 2.5 to 3.5 cm. broad, acute or acuminate at the apex and the base, on slender hirsute petioles of 3 to 5 cm., the latter semi-amplexicaul at the base, hirsute on both surfaces but denser below, 7- to 9-nerved; spikes hirsute, glabrous when old, flexuous, 30 to 45 cm. long; bracts and sepals ciliate, the bracts little more than half the length of the calyx, with a few hairs in the axils.

Kauai: "Tabular Summit," U. S. Explor. Exped. in Gray Herb.; Waimea (2,000–3,000 feet) Mann & Brigham 613, in Gray Herb.; Waimea (2,000–3,000 feet) Hillebrand, Berlin Herb.

Oahu: Makaleha Valley, Hillebrand, Berlin Herb., specimen not seen.

Variety *hirtella*, while distinct from other varieties, differs only slightly from var. *denticulata* from Molokai and that mainly in the permanent wool on the stem. There is quite a noticeable denticulation present on the leaf of the type specimen as well as on the specimens collected by Mann. The petioles in the present variety are very slender, while in those of Molokai they are broad and stem-clasping.

The distinguishing characters given by Hillebrand as "stem hollow in *hirtella* and solid in *denticulata*" do not hold good, since *denticulata* has also hollow stems or the central cavity is filled with a more or less spongy pith near the base of the stem which makes it appear to be solid.

Plantago princeps var. denticulata Hillebr. Fl. Haw. Isl. 364. 1888.

Stems 60 to 90 cm. long, with permanent scaly wool in the axils; leaves oblong-obovate, 15 to 25 cm. long, 3 to 5 cm. broad, not distinctly petiolate or the petioles broadly winged, broadly dilating at the base and semi-amplexicaul, hispid on both faces or papillose, 9- to 11-nerved, margins glandular-denticulate; numerous spikes, often 45 to 60 cm. long, hispid when young or glabrous when old, loosely flowered; bracts ciliate, but with very short indistinct hair in the axils; capsules as long as the calyx.

Molokai: Pali of Pelekunu, Hillebrand in Berlin Herb.; Pali of Waikolu, Hillebrand in Berlin Herb., College of Hawaii Herb. no. 16003, and Gray Herb.; Kamoku stream near camp, March 19, 1910, Rock, College of Hawaii Herb. no. 6120.

The stems of this variety are not simple, but branch in a candelabra-like manner; they are not solid as stated by Hillebrand, but hollow as is shown by his own specimens in the Berlin and Gray herbaria. Waikolu plants have more or less glabrous leaves, while those from Pelekunu are hirsute or hispid as are the writer's specimens from Kamoku stream, 1,500 feet lower than Pelekunu.

As has already been stated under variety *hirtella*, the present variety comes close to that variety, but differs in the glabrous stem and broadly winged or indistinct petioles. The glandular denticulation is the same in both varieties, but is less pronounced in the plants from Waikolu, which specimens are marked var. *hirtella* in Hillebrand's own handwriting in the Gray Herbarium.

The plants along the banks of Kamoku stream formed dense clumps or stands several meters in width. In the early spring of 1918 the writer revisited the exact spot, but not a vestige of the plants could be seen.

PLANTAGO PRINCEPS var. **anomala** Rock n. var. (Plate XIII.)

Plantago Queleana Heller in Minn. Bot. Studies **9**: 893. 1897.

Plantago princeps Heller ms. in Gray Herb.

Stem 120 cm. high, hollow, dividing at that point into five candelabra-like branches (*teste* Heller), with terminal leaf-clusters; leaves distinctly lanceolate to linear-lanceolate, about 30 cm. long, 3.3 to 4 cm. broad, bluntly acute at the apex, broadly sessile (15 mm. or more broad) at the base (petioles absolutely *wanting*), 11- to 15-nerved; nerves prominent on both sides, absolutely parallel and not converging, glabrous on both sides; spikes 50 cm. long, stout, glabrous; peduncle three-fourths the length of the leaves, densely flowered in the upper half; bracts longer than the calyx, acuminate; calyx segments oval, acute, half as long as the corolla; corolla tube long-exserted, the segments half the length of the part exserted; style glabrous or slightly pubescent; capsule nearly twice the length of the calyx, oblong, dehiscing at the middle; seeds (4) linear, shining, *black*.

KAUAI: Hanapepe valley, ridge opposite Gay and Robinson valley house, July 23, 1895, A. A. Heller, type, no. 2610 in Gray Herb.

This exceedingly interesting variety which Heller refers erroneously to *Plantago Queleniana* Gaud. (*Quelena* Heller), is almost worthy of specific rank. It differs from all other forms of *Pl. princeps* especially in the broadly sessile, very long, lanceolate leaves, with absolutely parallel veins, a character especially laid stress upon by Wawra in distinguishing the two species *princeps* and *pachyphylla*, and in the pyxidium, which is circumscissile at the *middle* and not at the base as in all other forms of *princeps*.

The characters which force us to place this anomalous plant as a form of *Pl. princeps* are the tall, branching stem and linear, oblong, black seeds, characteristic of that species.

Heller's misplacing of this exceedingly interesting plant must have been due to lack of material with which he could compare his specimens, but a careful analysis of the descriptions of the various forms of *Pl. princeps* should have convinced him that he had before him a plant of exceeding interest in so far as it is an intermediate between *Pl. princeps* and *Pl. pachyphylla*.

PLANTAGO PRINCEPS var. LONGIBRACTEATA H. Mann, Proc. Amer. Acad. **7**: 189. 1868.

Plantago princeps var. *aquatilis* inclusive of forma *erecta* Wawra, Flora **32**: 565–566. 1874.

Plantago Fauriei Lév. Report sp. nov. Fedde **10**: 151. 1911.

Plant herbaceous, drooping, fibrillous; stem very short, about 5 cm., pubescent and with long straight hairs at the leaf scars; leaves narrow-lanceolate, long-acuminate, pubescent above with scattered hairs, more or less abruptly narrowing at the base into a short winged petiole 3 to 3.5 cm. long, broadly subamplexicaul at the base, 7-nerved, the nerves covered with an ochraceous, matted, silky wool, especially in the young leaves; spikes less than twice the length of the leaves; peduncles about 7 to 8 cm. long, glabrous, loosely flowered, the ovate-sublanceolate bracts as long to twice as long as the corolla (11 mm.); sepals acute; flowers with long silky hairs at the base of insertion.

KAUAI: Hanalei, Mann & Brigham, 612, Gray Herb.; Hanalei et Hanapepe Waterfalls, Wawra, 2013*a*, 2013*b*, in herb. Vienna; Hanapepe falls, December, 1909, U. Faurie no. 1078 in herb. Léveillé.

A distinct variety, easily recognized by the long, subovate bracts and long acuminate leaves, whose veins are covered with densely matted, silky wool. It comes undoubtedly close to var. *acaulis*. Its stunted form is probably due to the habitat, as it enjoys the steep rock walls along waterfalls where it is exposed not only to the spray, but also to the force of the water itself.

Wawra records a plant from the same locality, not exposed to the force of water, but as growing in the open places and there developing longer and thicker stems and broader leaves, which he refers to a forma *erecta*. To the writer's mind this procedure is not permissible, because the latter habit of the plant is due to location only.

PLANTAGO PRINCEPS var. ACAULIS Wawra, Flora **32**: 564. 1874.

Root-stock about 15 cm. long, nodose, stem wanting; leaves at the apex of the caudex, densely woolly in the axils or with long, silky hair, oblong-lanceolate, acuminate, 12 to 18 cm. long, 2.5 to 3.5 cm. broad, gradually narrowing at the base into a distinct petiole 3 to 6 cm. long, 7-nerved, pale below, darker above, the nerves prominent below with a dark brown pubescence, margins entire or minutely denticulate in the upper half; spikes 1 to 4, densely flowered, glabrous, about 25 cm. long including a peduncle of 14 cm.; flowers hispid at the base or glabrous; bracts and calyx puberulous or glabrous; anthers oblong, apiculate, affixed at the middle to the very slender filaments, not exserted; capsule and seed as in the foregoing.

OAHU: on clay location, above Pali, Wawra, no. 1728*a*, in herb. Vienna. Koolau Mts., Punaluu, flowering, Dec. 24, 1908, Rock, no. 391 in the College of Hawaii Herb.; Koolau Mts., Punaluu, flowering and fruiting, June 11, 1916, O. H. Swezey, no. 16,005 in the College of Hawaii Herb.

This variety, while entirely stemless, seems to come close to *Pl. princeps* var. *Queleniana*. It also occurs in the rain forests as does Gaudichaud's *Queleniana*. It is distinguished by the leaves, which are dark above and pale below, and by the prominent nerves, which are somewhat hispid.

PLANTAGO PACHYPHYLLA A. Gray, Proc. Amer. Acad. **6**: 54. 1866.

Stemless; rootstock thickly covered with wool; leaves leathery, oval-oblong, ligulate-lanceolate, entire, 5- to 11-nerved, glabrous or tomentulous and puberulous, much shorter than the spike; spike elongate, densely flowered, the flowers woolly at the base (at last often glabrous); bracts and sepals ovate-obtuse or very obtuse; corolla lobes obovate, obtuse or very obtuse, or, after flowering, acute; ovules 2 to 4 in each locule.

PLANTAGO PACHYPHYLLA var. α MAUIENSIS Gray, Proc. Amer. Acad. **6**: 54. 1866.

Caudex very thick, densely woolly with long, silky tomentum; leaves broad, 12.5 to 26 cm. long, 3 to 9 cm. broad, ovate, acute at the apex, 9- to 11-nerved, glabrous above, covered with a deciduous wool beneath, veins dark brown, conspicuous below, parallel, arcuate; petioles 2.5 to 15 cm. long, very broad (2.5 cm. or more at the base), the blade gradually narrowing into a short or long, broad petiole; spikes numerous, robust, densely woolly, terete, up to 82 cm. long, 8 mm. in diameter; peduncle as long as or longer than the leaves; rachis shorter than the peduncle; flowers arranged at short intervals in the upper half, woolly at the base; bracts and sepals ovate-obtuse or shortly ovate, with a broad, black median nerve, often pubescent; corolla lobes obtuse or acute; stamens and style long-exserted; ovules in each locule 2 to 4, capsule dehiscing at the middle, obtuse at the apex; seeds oval, dark-brown, almost blackish.

MAUI: U. S. Explor. Exped. on Mauna Haleakala (7,500 feet), type in Gray Herb.; Mann & Brigham, Haleakala, no. 428 Gray Herb.; Wawra, N.E. side Haleakala, no. 1912 Vienna Herb., spec. n.v.; Hillebrand, Haleakala, 6,000–8,000 feet, Berlin Herb., spec. n.v.; Puu Nianiau, slopes of Haleakala (7,000 feet), Rock, September, 1910, no. 8555 in the College of Hawaii Herb. (Under the latter number there are two specimens, one with shorter leaves but not oval, and resembling Mann's no. 428, which must undoubtedly be referred to this variety. The other specimen has much longer leaves with long, very broadly winged petioles, the spikes are very robust, and the sepals and bracts are pubescent while in the typical form these are glabrous.)

PLANTAGO PACHYPHYLLA var. MAUIENSIS forma **montis eeka** Rock forma nova.

Caudex as in *P. mauiensis;* leaves obovate-oblong or ovate-oblong, gradually narrowing into a very broad, winged petiole, up to 10 cm. long, 2.5 cm. broad at the base; leaves densely matted below with thick, brown wool, with the exception of the clasping base of the petiole, nerves indistinct; spikes shorter than in *P. mauiensis*, robust, densely flowered and covered with matted wool; flowers embedded in the more or less deciduous wool; bracts and sepals oblong, acute or obtuse, as long as or longer than the corolla tube, the broad median nerve densely woolly; corolla lobes short, acute, and glabrous; anthers glabrous, exserted.

WEST MAUI: Slopes of Puu Kukui (6,000 feet), flowering and fruiting, Aug. 21, 1910, Rock, no. 8213 in the College of Hawaii Herb.

(It grew in company with another form with leaves perfectly glabrous beneath, no. 8214.)

This form differs from the typical *mauiensis* in the leaves, which are densely matted with wool beneath instead of being tomentose, and in the nerves, which are consequently indistinct beneath. The median nerve of the calyx is also covered with wool, and the stamens are broader than in the type.

PLANTAGO PACHYPHYLLA var. HAWAIIENSIS A. Gray, Proc. Amer. Acad. **6**: 54. 1866. Inclusive of subvariety *gracilis* A. Gray l.c. 55.

Caudex more or less woolly; leaves ovate-lanceolate, broadly lanceolate, linear-ligulate, or lanceolate-oblong, 3- to 9-nerved, narrowed at the base into a very short or slender petiole, 1.5 to 6 cm. long; spike up to 40 cm. long, glabrous or with deciduous pubescence, slender, loosely flowered; sepals nearly all ciliolate, slightly shorter than, or twice the length of, the capsule; capsule oblong to ellipsoidal, 4- to 6-seeded.

HAWAII: Mauna Kea and Mauna Loa (6,000–8,000 feet) "in the environs of the great crater," Remy, 1851–5, no. 429 in Gray Herb.; on lava bed near Kalulu, Mt. Hualalei (6,000 feet), June 10, 1909, Rock, nos. 3722 and 3672 in the College of Hawaii Herb.

Subvariety *gracilis* is not distinct enough to be retained as a subvariety, but must be included in var. *hawaiiensis*. The writer's plants from Hualalei agree very well with Remy's specimen no. 429, but the leaves also agree with Gray's typical *hawaiiensis*. On the same sheet with *hawaiiensis* is a pubescent specimen which Gray marked inter α and β. It is evidently a pubescent form, the pubescence disappearing in older leaves. Hillebrand's specimen belongs to that form. Hillebrand's specimen from Mt. Eeke marked var. *hawaiiensis* does not belong here, but undoubtedly belongs to var. *kauaiensis*, as it agrees fairly well with the type of that variety.

PLANTAGO PACHYPHYLLA var. KAUAIENSIS A. Gray, Proc. Amer. Acad. **6**: 55. 1866.

Herbaceous, caudex very short, fibrillous, woolly between the leaves; leaves coriaceous, linear-lanceolate, 5 to 6 cm. long including the very short, broad, sessile petiole, obtuse at the apex, rugose above, 3- to 5-nerved, impressed above, glabrous, pubescent beneath or glabrate; spikes one to several, slender, black, glabrous or hirsute with brownish hairs in the young state, loosely flowered; peduncle slender, 9 to 14 cm. long; rachis of the same length or slightly longer; flowers partly woolly at the base; bracts shorter than the calyx, obtuse; calyx segments obtuse or subacute; corolla segments ovate, acute; anthers broadly ovate, apiculate; style long-exserted, pubescent; capsule exceeding the calyx, dehiscing at the middle, often 1-seeded; seeds oval, pale brown, never viscous.

KAUAI: On tabular summit, U. S. Explor. Exped., type in Gray Herb.; Waialeale, Oct. 20, 1911, Rock, no. 8891 in College of Hawaii Herb. (No.

8891*b* from the same locality differs from 8891 mainly in the hirsute spike.)

OAHU: Konahuanui, Feb. 22, 1914, O. H. Swezey, no. 12771 College of Hawaii Herb. (Nerves on under side of leaves slightly hairy.)

PLANTAGO PACHYPHYLLA var. KAUAIENSIS forma **robusta** Rock forma nova.

Caudex thick, long, creeping; leaves thick, coriaceous, linear-lanceolate, of nearly even width, broadly sessile at the base, 5 to 17 cm. long, up to 3 cm. broad, 7- to 9-nerved, glabrous on both sides, acute at the apex; spike 22 to 58 cm. long, stout, terete, glabrous or puberulous; bracts and sepals acute, glabrous or puberulous, especially on the median nerve; capsule obtuse, as long as the calyx, 2-seeded.

WEST MAUI: Summit of Mt. Eeke, Hillebrand in Gray Herb. and in College of Hawaii Herb. ex Herb. Berlin; summit of Mt. Eeke, August, 1918, Rock, no. 16006 in College of Hawaii Herb.

(The W. Maui plants referred by Hillebrand to *hawaiiensis* cannot be separated from var. *kauaiensis*, but differ in the much stouter, terete spikes. The writer's specimens from Mt. Eeke have slender spikes as in the typical *kauaiensis*, but are pubescent and glabrous afterwards and, in some instances, floriferous almost to the base.)

MOLOKAI: Kawela Swamp below Pelekunu, growing with Oreubulus and Panicum, March 17, 1910, Rock, no. 6098 in College of Hawaii Herb.

(Although the Molokai plant is less robust than the Maui form it cannot well be separated from it. The leaves are slightly pubescent on both surfaces and more flaccid. The leaves are glandular-denticulate and somewhat pubescent, especially on the nerves.)

The hairiness of the spike is evidently an unreliable character, as hairy and glabrous spikes occur on identical plants.

The writer's specimen (no. 8214) from Puu Kukui, West Maui, are identical with those of Hillebrand from Eeke. It is exceedingly doubtful if Hillebrand had ever visited Mt. Eeke, which doubt seems to be confirmed by the fact that he makes no mention of the thousands of silverswords occurring there and which are practically absent from Puu Kukui.

PLANTAGO PACHYPHYLLA var. KAUAIENSIS forma **intermedia** Rock forma nova.

A specimen collected on Waialeale, Kauai, no. 8891*a*, with hirsute spikes and leaves hirsute on the upper surface, is intermediate between *kauaiensis* and Wawra's variety *pusilla*.

Plant larger, leaves linear-oblong, glabrous and prominently nerved below, strigosely hispid above, but finally deciduous with the exception of the apices of the leaves; spikes shorter, covered with deciduous hairs, rachis longer, angular; calyx segments acute, otherwise as in var. *pusilla*.

KAUAI: Waialeale, Oct. 20, 1911, Rock, no. 8891*a* in College of Hawaii Herb.

Forma *intermedia* differs from the typical *pusilla* in being a larger plant, in the more robust spikes, which are many-flowered and hirsute, and in the acute sepals. It differs from *kauaiensis* mainly in the linear-oblong leaves, which are strigosely hispid toward the apices, and in the shorter spikes. It forms the transition type between varieties *kauaiensis* and *pusilla*.

PLANTAGO PACHYPHYLLA var. PUSILLA Wawra, Flora 33: 568. 1874.

Herbaceous, rosette-like; caudex very short, woolly between the leaves; leaves numerous, coriaceous, 2 to 4 cm. long, 4 to 6 mm. broad, oblong, spathulate, acute or rounded, 3- to 5-nerved; blades glabrous below, strigosely hirsute above with yellow articulate hairs, entire; spikes (1 to 7) 4 to 15 cm. long; peduncle glabrous, shining, slender, floriferous on its upper third or fourth and bearing from 3 to 5 flowers; rachis glabrous, but flowers woolly at the base; bracts half the length of the calyx, glabrous, rounded; sepals rounded, glabrous; corolla lobes linear-oblong, membranous, obtuse, one fourth the length of the tube; capsule shortly acute, dehiscing at the middle, 4-seeded; seeds reddish-black, oblong or ovoid-oblong, minutely wrinkled.

KAUAI: Plateau of Waialeale, no. 2166 Wawra, in Herb. Vienna, spec. n.v.; Waialeale, Oct. 20, 1911, Rock, no. 8890 in College of Hawaii Herb.; Waialeale, Oct. 20, 1916, Rock, no. 16007 in College of Hawaii Herb.

No. 16007 seems to be the typical *pusilla*, while no. 8890 is somewhat larger, the leaves being woolly below, at least along the nerves.

PLANTAGO PACHYPHYLLA var. ROTUNDIFOLIA Wawra, Flora 32: 567. 1874.

Caudex very short, fibrillous; leaves rosette-like, coriaceous, obovate or almost orbicular, 5 to 7.5 cm. long, narrowing at the base but not really petiolate, glabrous above, covered with a thick ochraceous stuppeus indumentum, 5-nerved; nerves arched, impressed above, invisible below; spikes one or two, erect, covered with a silky pubescence, rachis shorter than the peduncle, densely flowered; flowers woolly at the base, at length naked; bracts obtuse; calyx segments obtuse; corolla lobes broadly ovate, obtuse; capsule equaling the calyx, circumscissile at the middle, bilocular, 2-seeded; seeds broadly ovate, yellowish brown, never glutinous.

KAUAI: Waialeale, high plateau in moss on trunks of trees, Wawra, no. 2201 in herb. Vienna, spec. n.v.

PLANTAGO PACHYPHYLLA var. ROTUNDIFOLIA forma **crassicaudex** Rock forma nova.

Caudex very thick, 10 cm. or more long; leaves obovate-oblong, with revolute margins, densely woolly below as in the variety, glabrous above; otherwise as in variety *rotundifolia*.

MAUI: Mt. Eeke, August, 1918, Rock, no. 16008 in College of Hawaii Herb.

The writer establishes this form rather reluctantly as there are no mature spikes present on the single specimen collected, and only dead spikes remain. It differs, however, sufficiently from variety *rotundifolia*, especially in the thick 10-cm. long caudex and in the obovate-oblong leaves.

PLANTAGO PACHYPHYLLA var. GLABRIFOLIA Rock, Indig. Trees Haw. Isl. 77. 1913.

Caudex very short, thick, and matted with wool; leaves forming large rosettes, broadly ovate in outline or ovate-oblong, acute, thick coriaceous, glabrous on both surfaces, broadly sessile at the base, 9- to 11-nerved, the nerves prominent below, the lateral ones arcuate, margins inconspicuously glandular-denticulate, 15 to 18 cm. long, 5.5 to 10 cm. broad; spikes 1 to 4, stout, glabrous or pubescent in the young stage, densely flowered in the upper third, flowers crowded toward the apex, loosely flowered toward the base of the rachis; bracts as long as the calyx, obtuse; sepals acute; corolla lobes acute or obtuse, reflexed; style long-exserted, gray-hairy; anthers more or less excised at the base, oblong to ovate; capsules unknown.

KAUAI: Waialeale, Oct. 20, 1911, Rock, no. 8889 in College of Hawaii Herb.

This variety differs from the others in the very broad, almost orbicular leaves which are glabrous on both surfaces, and in the glabrous spikes which are densely flowered, the flowers being oblong in outline rather than oval. With the exception of its glabrousness it would be referable to the typical *Plantago pachyphylla* var. *mauiensis*.

Variety *kauaiensis* and its forma *robusta* from Maui and Molokai undoubtedly are very closely related and are perhaps only forms of *Pl. pachyphylla* var. *mauiensis*, although large-leaved glabrous forms grow together with hirsute, slender and robust forms in the same locality.

PLANTAGO PACHYPHYLLA var. **muscicola** Rock. var. nova.

Caudex short, thick, densely fibrillous below, the roots densely hairy as is the whole plant; leaves ovate to ovate-oblong, brittle, thick, fleshy, about 2 cm. or more in thickness, including the dense, gray hairs on both surfaces, these sometimes at right angles to the blade, 15 to 20 cm. long, 5 to 8 cm. wide, acute at the apex, contracted below but broadly sessile at the base, 7- to 9-nerved; nerves inconspicuous owing to the pubescence, arcuate; margins of the leaves more or less conspicuously glandular-denticulate; spikes numerous (up to 10), densely hairy, stout, terete; bracts as long as the calyx or longer, hairy; sepals broadly ovate, acute, ciliate at the apex; corolla lobes broadly oval, acute, 1-nerved; capsule exserted, oblong, obtuse, 2-seeded; seeds oval, dull brownish, rounded at both ends.

HAWAII: Below summit of Kohala Mts. back of Waimea, elevation 4,200 feet, in open bog, embedded in thick sphagnum, June, 1910, Rock, no. 8315 in the College of Hawaii Herb.

This interesting variety differs from all the others in the thick, brittle, fleshy leaves which are densely hirsute on both surfaces with long, gray hairs which stand at right angles to the blade. The numerous spikes are very robust and hirsute as are the leaves. Otherwise as in *Plantago pachyphylla*. Specimens of this variety have been distributed to herbaria as *Plantago muscicola*.

INTRODUCED SPECIES

PLANTAGO MAJOR L. Sp. Pl. **1**: 112. 1753.

HAWAII: Makahalau, Parker Ranch, June 23, 1909, Rock, no. 3146 in College of Hawaii Herb.

OAHU: Pauoa Valley, Oct. 24, 1908, Rock, in College of Hawaii Herb.

This species is now distributed over all the islands along roadsides and pastures. It is a native of Europe and Asia. On Hawaii the largest specimens have been collected. It is of early introduction.

PLANTAGO LANCEOLATA L. Sp. Pl. **1**: 113. 1753.

HAWAII: Waikii, 5,000 feet elevation, July, 1909, Rock, in the College of Hawaii Herb.

KAUAI: Hanapepe river basin, June 28, 1895, Heller, no. 2457 in the College of Hawaii Herb.; Kaholuamano, July, 1909, Rock, no. 5728 in the College of Hawaii Herb.

This species, also a native of Europe, made its appearance in the Hawaiian Islands only within the last 25 years or so. It was first observed on the island of Kauai. It was introduced with impure grass or flower seed by Gay and Robinson from Australia, where the plant has been proclaimed. It is abundant at Kaholuamano (elevation 3,400 feet), where it even crowds out grasses.

PLANTAGO VIRGINICA L. Sp. Pl. **1**: 113. 1753.

HAWAII: Parker ranch, June 24, 1909, Rock, nos. 3138 and 3139 in the College of Hawaii Herb.

This species is a native of North America, where it occurs from Rhode Island to Florida and has also been collected in the Bermudas. On Hawaii it is very common on the pasture lands of the Parker ranch, especially at Kanahiokaoka in Mana, and Paauhau, at an elevation of about 3000 feet. Some of the paddocks are completely taken possession of by this species, the rosettes of which are flat on the ground and not erect.

DOUBTFUL SPECIES

PLANTAGO GAUDICHAUDIANA Lév. Rept. Sp. nov. Fedde **10**: 151. 1911.

"Pulcherrima species his notis facile diagnoscenda; stirpe foliorum emortuorum vaginis foliis laceratis obtecta; scarpa striato glabro circa 70 cm. alto, virgato, erecto; folia radicalia glabra 5 nervia, 20 cm. circiter longa et 1 cm. lata nec dilatata; spica 30 cm. alta; flores numerosi, dissiti, sparsi, subverticillati; bracteis concavis, brunneis, acuminatis; sepala nigro-brunnea margine scariosa obtusata style brunneo exserto.

"Haec est, mea sententia, illa planta, cui speciminibus maximis visis, Gaudichaud nomen nudum *quelea* imposuit."[1]

HAWAII: Mauna Kea, 2,000 meters, June, 1909 (Faurie, no. 1075).

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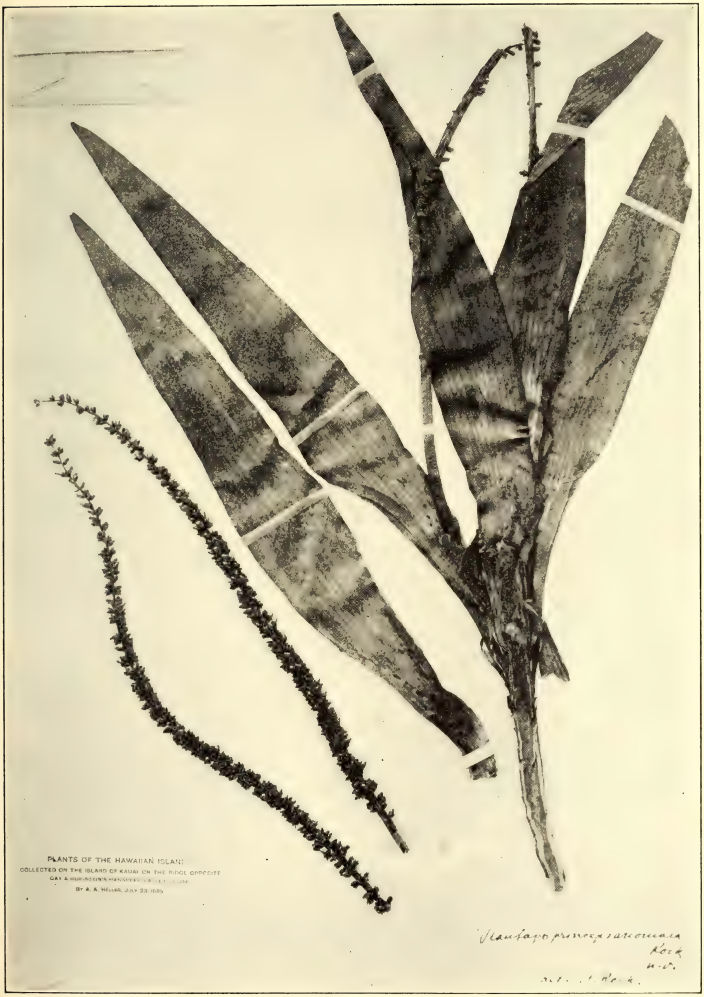
[1] In the writer's opinion the plant is referable to one of the many forms of *Plantago pachyphylla*, probably to var. *hawaiiensis* Gray.


PLANTS OF THE HAWAIIAN ISLANDS
COLLECTED ON THE ISLAND OF KAUAI ON THE RIDGE OPPOSITE
BY A. A. HELLER, JULY 23 1895
Plantago princeps var anomala
Rock
n.v.

ROCK: PLANTAGO PRINCEPS VAR. ANOMALA ROCK. PHOTOGRAPH OF TYPE IN THE GRAY HERBARIUM.

RELATION OF CATALASE, OXIDASE, AND H^+ CONCENTRATION TO THE FORMATION OF OVERGROWTHS

R. B. Harvey

Overgrowths were found by the author (7) to result from local freezing of the leaf tissue in a number of plants, including cabbage (*Brassica oleracea capitata*) and *Bryophyllum calycinum*. These overgrowths offer special opportunity for the separation of certain factors concerned in the rejuvenescence of cells, and for the comparison of physiological conditions attendant upon the production of overgrowths resulting from infection with *Bacterium tumefaciens* and from the stimulation of normal tissue by physical and chemical means. The literature of overgrowths as a result of bacterial infections in plants has been fully presented in papers by Dr. Erwin F. Smith.

The economic importance (9, 19, 20) of certain plant diseases in which overgrowths are produced, and the relation of these to growths of similar nature occurring in man and other animals (3, 4, 5), have had sufficient discussion in recent articles (6) to make comment unnecessary in this paper. The production of intumescences by means in which bacteria are not concerned has been noted recently by Smith (2) and Wolff (1).

In a paper by Dr. Erwin F. Smith (2, p. 167) it was suggested that osmotic relations between tumor and healthy tissues might offer an explanation for overgrowths. With a view to determining these osmotic relations the author has determined the freezing points of tumor and healthy tissues in different plants. It now appears that determinations referred to in a publication by Dr. Smith (3, p. 441) may be in error on account of the difficulty in obtaining the true freezing point of the tissues from the freezing point of the expressed juice.

The freezing point of tumor and healthy tissues taken from the same plants was obtained by expressing the juice with a Buchner hand press, and with an hydraulic press using 10 tons on a 2½ inch ram.

The freezing point of juices expressed from a tissue vary according to the treatment before expression and also according to the pressure applied (7, p. 94). Also, it appears that variation in the quantity of wood in the tissue prevents one from obtaining a uniform sample by pressure.

In table 1 are given the freezing points of juices expressed after freezing with solid CO_2 and grinding in a mortar while frozen dry. Stem tissue was taken from nodes immediately adjacent to the tumors. The tumor material was supplied by Dr. Smith's laboratory and was produced by inoculation with *Bacterium tumefaciens*.

Table 1. *Freezing points of juices from tumor and healthy tissues*

	Ricinus	
Leaf	− 0.793° C.	− 0.815° C.
Tumor	− 0.953 C.	− 0.746 C.
Stem	− 0.712 C.	− 0.590 C.
	Daisy	
Leaf	− 0.810° C.	
Tumor	− 1.170 C.	
Stem	− 0.783 C.	
	Beet	
Leaf	− 0.920° C.	
Tumor	− 0.970 C.	
Root	− 1.210 C.	

In Ricinus and daisy the tumor tissue yields an expressed juice with a greater freezing-point depression than that of adjacent normal tissue. But there is more woody tissue in the normal stem than in the tumor tissue of the Ricinus and daisy. The author does not regard these freezing points as the true values for the tissue. They are given for the purpose of showing the errors which may arise from the method of obtaining the freezing points of these tissues.

By using a thermocouple threaded through the tissue the freezing point can be obtained directly. The apparatus used in these determinations was the same as that reported in a previous paper (18). A piece of tissue of the same size in each case was threaded upon the thermal junction and cooled by ether evaporation to a desired point. Inoculation of the tissue was brought about by knocking it against the wall of the surrounding tube which was covered with frost. By this means the undercooling could be regulated quite accurately. When the undercooling is the same, the freezing points of the tumor tissue and of adjacent healthy tissue are nearly the same; in any case there is only a few hundredths of a degree difference, as shown in table 2.

Table 2. *Freezing points of tumor and healthy tissues obtained by thermocouple*

	Undercooling	Freezing point
	Ricinus	
Tumor	− 1.49° C.	− 0.40° C.
Healthy stem	− 1.49 C.	− 0.41 C.
Tumor	− 2.20 C.	− 0.60 C.
Healthy stem	− 2.37 C.	− 0.64 C.
	Beet	
Tumor	− 2.92° C.	− 1.38° C.
Healthy root	− 2.90 C.	− 1.33 C.
Tumor	− 4.44 C.	− 1.74 C.
Healthy root	− 4.46 C.	− 1.72 C.

These values are free from errors arising in expression of the juice and represent the true freezing points of the tissue. They are of greater value than those made on expressed sap because small pieces of tissue can be taken immediately adjacent. Since such good checks were obtained, the author is inclined to believe that there is but little difference in the osmotic concentration in these particular cases.

Juices expressed from tumors produced by inoculation with *Bacterium tumefaciens* show a hydrogen-ion concentration consistently a little lower than that of juices expressed from healthy stem tissues taken from adjacent nodes, as shown in table 3.

TABLE 3. *H^+ concentrations of tumor and healthy tissues*

	Ricinus P_H	$C_H \times 10^6$
Tumor juice	5.822	1.51
Healthy stem juice	5.411	3.88
Leaf juice	5.580	2.63
Tumor juice	5.886	1.30
Healthy stem juice	5.817	1.52
Leaf juice	5.739	1.82
Tumor juice	5.62	2.40
Healthy stem juice	5.35	4.47
Leaf juice	5.48	3.30
	Beet	
Tumor juice	6.347	0.42
Healthy root juice	5.818	1.52

These tumors were in actively growing condition. The juice was expressed after grinding in a meat chopper but without freezing the tissue, since the hydrogen-ion concentration of a juice has been shown to be changed by the precipitation of the proteins on freezing the tissue. The determinations were made by the potentiometric method.

It may be suspected that the expressed juice will show a H^+ concentration different from that of the cell sap of the vacuoles owing to errors arising by expression. This may be the case in some tissues. However, the author has been able to dilute the juice from such tissues as tomato fruit to one fifth the original concentration without appreciably changing the H^+ concentration. The H^+ concentration within the uninjured cells can be estimated only in tissues which have natural indicators.

The concentration of the buffer salts present in the tissue will determine whether or not they are able to maintain the original P_H value on dilution. Precipitation of globulins on too great dilution of the juice may bring about H^+ changes. The H^+ concentration of a buffer solution depends (within fairly wide limits) upon the ratio of the buffer substances present, and not upon their total concentration. In obtaining the freezing-point depression, the total concentration of the expressed juice must be the same as that within the tissue to obtain a true value; for the H^+ concentration this is not necessarily the case.

Although the differences in H^+ concentration shown in the above table are small, they may be of relatively great importance for the activity of the respiratory enzymes associated with growth. The curve for the activity of catalase of plant origin at various H^+ concentrations is practically the same as that for catalase of animal origin given by Michaelis (8). From this curve it will be seen that the H^+ concentration shown by the plant tissue lies in a critical region for the activity of catalase. A change from P_H 5.52, the average value for healthy stem tissue of Ricinus, to P_H 5.78, the average value for tumor tissue, increases the catalase activity 25 percent at an acetate concentration of fiftieth normal. The activity of oxidase is increased also by a decrease in the H^+ concentration. In a paper cited above (7, pp. 98–101) the author gave indications of the decrease in H^+ concentration resulting from freezing of leaf tissue. Areas of Bryophyllum leaves which contain anthocyanin are changed from red to blue on freezing. This is not necessarily accompanied by death of the tissue.

It is shown from the work by Dr. Smith and others (9, p. 113) that *Bacterium tumefaciens* blues litmus milk and decreases the H^+ concentration of the culture medium. This it appears to do also in the tissue in which it grows.

Since catalase is destroyed at increasing rates with increase in the hydrogen-ion concentration, it is of interest to compare the catalase activity of two tissues of the same plant which were the same before overgrowth was induced by stimulation but which finally have different hydrogen-ion concentrations. For this purpose, Ricinus plants were inoculated with *B. tumefaciens* and when the tumors had become sufficiently large they were removed. Healthy stem tissue was taken from the same node. Fifty grams of tissue were ground in a mortar with crushed quartz and 25 cc. of phosphate buffer mixture C_H 2.7×10^{-8}, and made up to 500 cc. with distilled water. The Van Slyke apparatus for amino-acid determination was found convenient for catalase determination when used in a constant temperature room. Five cc. of hydrogen peroxide (Oakland 3 percent neutral) was run into the reaction chamber of the apparatus and washed down with 10 cc. of distilled water. After adjusting the level in the measuring pipette, 10 cc. of plant tissue dilution was run in from the side burette. The apparatus was shaken only fast enough to give a good mixing of the solution, and the rate of shaking was kept the same throughout. After 10 minutes the following amounts of oxygen were evolved at 30° C.

Tumor tissue	44.6 cc. O_2	42.3 cc. O_2
Healthy stem tissue	13.2 cc. O_2	13.3 cc. O_2

Bryophyllum leaves were inoculated with *B. tumefaciens* by injecting a suspension of the bacteria into the leaves by means of vacuum. This produced rather large tumored areas. Healthy leaf tissue which showed no tumor formation was taken from the same leaf. Three and three tenths grams of tumor and healthy tissue were diluted to 50 cc. after treating as before. Twenty-five cc. of these dilutions gave in the same time for tumor tissue 38.9 cc. O_2; and for healthy leaf, 2.4 cc. of oxygen.

Intumescences were also induced on Bryophyllum leaves by freezing the plants at a temperature of − 2° C. After about 15 minutes it was observed that frozen areas appeared over the surface of the leaf. The plants were removed from the cold chamber when the areas were about 5 mm. in diameter and placed in the greenhouse. After five days the areas had grown into small intumescences. While these were still actively growing, leaves were selected in which the tumors formed about half the area. It was found impossible to get a greater percentage of the area to grow out as intumescences because death occurred if more than about half the leaf surface was frozen. The tumor spots were so small and so intermingled with healthy tissue that a quantitative separation of the tumored and healthy areas could not be made without introducing great error.

Six grams of leaf tissue of which the tumored areas were estimated to represent half the tissue were taken and compared for catalase activity with 6 grams of normal tissue from a leaf of the same age and size from the same plant. The tissues were ground with quartz and excess $CaCO_3$ and made up to 200 cc. Twenty-five cc. of the dilution were taken with 5 cc. of the hydrogen peroxide. After 15 minutes the following amounts of oxygen were evolved:

Tumor 50 percent	3.1 cc. O_2
Healthy tissue	1.05 cc. O_2

Tissues of beet in which the overgrowths were produced by inoculation with *B. tumefaciens* gave on treatment in the exact manner given above for Ricinus tissue:

Tumor tissue	8.1 cc. O_2	8.9 cc. O_2
Healthy root tissue	5.6 cc. O_2	5.4 cc. O_2

It should be noted that the increase in catalase activity of the intumescences produced no Bryophyllum leaves by *Bacterium tumefaciens* is much greater than that of the intumescences produced by freezing.

The peroxidase and catalase activities in intumescences of cabbages induced by freezing are greater than those in normal tissues of the same leaf.

Since this decrease in H^+ concentration increases the catalase activity, it is interesting to note that the catalase activity is greatly decreased in mosaic leaves of tobacco which show greater H^+ concentration than healthy leaves. In this case also, the P_H values are but slightly different, yet it appears of great physiological importance for the growth of the mosaic-diseased cells.

The oxidase activity of tumor and healthy tissue was obtained on beets inoculated with *B. tumefaciens*. Tissue dilutions representing the same green weight of material were made up to C_H 3.6 × 10^{-7} and $n/60$ total salt concentration. Oxidase determinations were made in an oxidase apparatus which has been described in another place (10) and which is a modification of the simplified Bunzell (11) oxidase apparatus (12). After shaking for four hours at a constant temperature of 30.2° C., the following readings were given as the average of three determinations:

Oxidase activity of beet tissue

Tumor tissue	
Hydrochinone	3.24 cm. Hg.
Pyrogallol	4.14 cm. Hg.
Healthy tissue	
Hydrochinone	0.90 cm. Hg.
Pyrogallol	1.70 cm. Hg.

In this case it appears that the oxidase activity in the tumored areas is greatly increased. An increase in oxidase activity in tumored tissues has been reported in a number of articles (22).

In this connection it is interesting to note that the anthocyanin color is greatly increased in the tumored areas of Bryophyllum leaves induced by freezing, so that the intumescences stand out as red areas. The intumescences are produced mostly along the veinlets rather than in the vein islets. The deepest color of anthocyanin is located along the veinlets within the intumescences.

When Bryophyllum leaves are frozen in spots over a small percentage of the area, the frozen spots turn brown. One can predict from the depth of this brown color whether the area will die or will be stimulated to growth. As the percentage of the total area which is frozen increases, the depth of brown color in the spots decreases. When the whole leaf is frozen the color is uniformly distributed. If brown-spotted leaves are killed by freezing or by ether, the spotted areas maintain the deepest color, showing that the oxidizable substances have been partly removed from the surrounding tissues. It appears from this that there is an accumulation of the colored compounds within the frozen areas. This becomes very marked about 12 hours after freezing, as shown in the photograph (fig. 1). The greatest development of color occurs along the veinlets within the frozen areas (fig. 2). The frozen areas seem to have the property of taking up substances from the surrounding tissues which are converted within them and which accumulate as brownish colored compounds. Onslow (13) has given us data on the nature of such substances. Substances of the type of catechol occur in plants which brown on injury. Peroxidase activates the oxidation of these aromatic compounds, and the oxidized product is an organic peroxide.

It has been shown by Krassnosselsky (14) that an increased rate of respiration follows frost injury. The author so far has not been able to compare the respiratory rate of the frozen and normal areas. It would be desirable also to determine the oxygen acceptors in these spots. There is no immediate increase in the catalase activity of the areas frozen, nor does this increase until growth can be observed. Blackening indicates that there is an abnormally oxidized condition in the frozen areas.

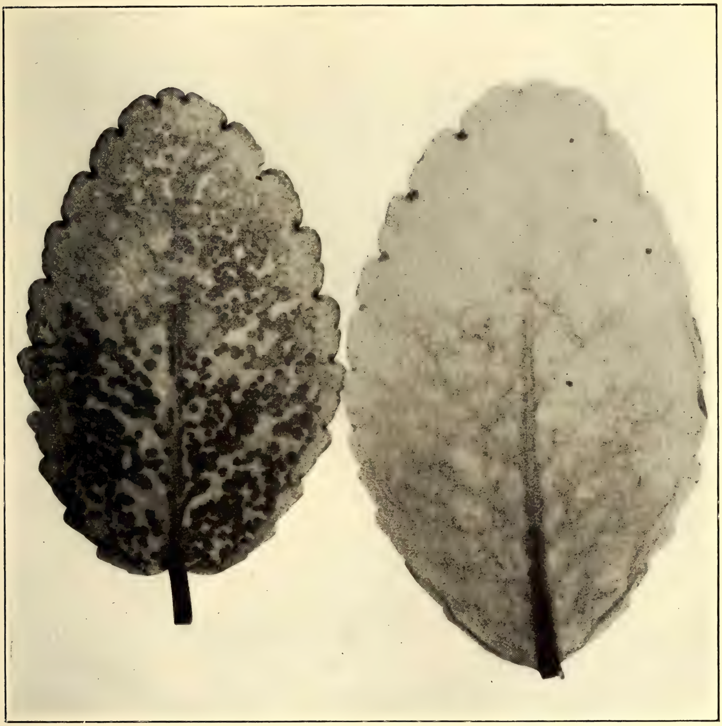


FIG. 1. Development of color in Bryophyllum leaves as a result of freezing. Notice the deep color in the small isolated frozen areas and the decrease of the color intensity with increasing size and frequency of the spots. The leaf with uniform color distribution was frozen throughout.

DISCUSSION

To the author it seems plausible that the hydrogen-ion concentration of the cell sap in the frozen areas may be decreased by removal of H^+ during the precipitation of proteins, which has been shown by the author to occur on freezing (7, p. 103). This decrease of H^+ concentration favors the activity of catalase and oxidase. The author has shown (7) that in hardened cabbage leaves there is no production of intumescences from frozen spots. The reason ascribed for this was that there was no permanent combination of protein and H^+ and hence no H^+ change or protein precipitation in this case. There may be attendant upon the protein precipitation, which could occur especially at the outer boundary of the protoplast, an increased permeability of the membranes for oxygen, so that the oxygen concentration within the cell is increased. The next attendant condition would be the formation of increased quantities of organic peroxide. The oxidation proceeds to the formation of colored compounds, finally producing substances such as purpurogallin from pyrogallol or melanins from tyrosine. The oxydo-reductases are prevented from normally reducing these colored compounds by the increased concentration of oxygen in the cells (15).

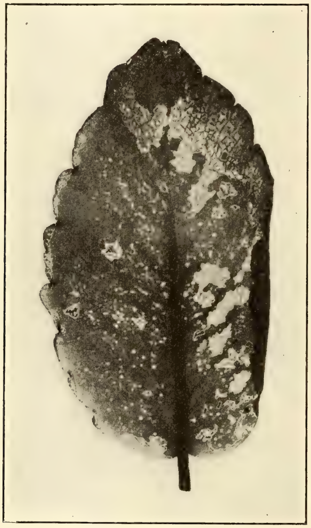


FIG. 2. Intumescences on Bryophyllum leaf induced by freezing. Notice occurrence of intumescences along the veinlets and the brown color along the veins in the light-colored areas. Light-colored areas are due to death of tissue because too great a percentage of the area was frozen. Local freezing occurred in these areas along the veins only, and brown color developed there. Subsequently the cells in the light-colored areas died. Renewed growth is not due to isolation, for three islets of normal tissue occur in the upper portion of the leaf and these show no renewed growth.

If the cell has been injured to such an extent as to be killed, all the oxygen acceptors are changed to the final state of equilibrium with the air. In case the cell recovers, an increased rate of oxidation has been established in it above the rate of oxidation in the cells in areas not frozen, owing to the greater concentration of organic peroxide and decreased H^+ concentration.

Child (16) has shown that dominance of growth is conditioned by a higher rate of metabolism in the growing region than in the surrounding area. When this increased rate is once established, it then opens the way for further growth at the expense of the surrounding tissue. In case too great a percentage of the leaf cells are frozen, there is no great concentration and transformation of the oxygen acceptors in any one area, as shown by little development of brown color. Hence there is little growth produced, because no one frozen area dominates a sufficiently large area of normal tissue which supplies it. This is actually the case. The most rapidly growing areas are small and somewhat scattered. Loeb (17) has suggested that the quantity of growth in *Bryophyllum calycinum* is conditioned by the quantity of some growth-promoting substances within the leaf. It is entirely possible that the usual sources of energy, the carbohydrates, are not the only substances concerned, but also that there are chromogenic substances of equal importance. Oxidase determinations indicate that there may be such substances produced or accumulated in plant tumors caused by *Bacterium tumefaciens*.

Loeb (21) also suggests that the dominance of a growing apex is due to the production within it of inhibiting substances which hold in check the neighboring buds. The difficulty with this assumption is that if the inhibiting substance is produced in the dominant apex it should depress growth there also since it would be in greatest concentration there. This ought to be a general objection to such assumptions. It seems to the author more plausible to assume that in correlation of growth the dominance of a growing area is conditioned not by the production of an inhibitor for the area around it, but by the removal from the surrounding area of growth-stimulating substances and their accumulation in the dominant area. These growth-stimulating substances, such as the chromogens of Palladin (23), are produced by all the cells and are diffusible. We have seen in a case cited above (*Bryophyllum*) how they accumulate in a frozen area and are associated with a renewal of cell growth.

In the case of inoculation of tissues with *Bacterium tumefaciens*, the presence of this bacterium favors the action of the respiratory enzymes by locally decreasing the H^+ concentration. This condition is continuous, and hence the growth process should be continuous. The bacteria in such tumor tissue evidently do not produce substances detrimental to cell multiplication, but may produce substances which favor oxidation within the tissue.

SUMMARY

It was found that the concentration of osmotic substances was the same in tumor and healthy tissues in Ricinus and beet when measured by the thermocouple method. Hence osmotic relations do not account for the tumor production by *Bacterium tumefaciens* in these cases.

Catalase, oxidase, and peroxidase activity is greater in tumor tissue than in adjacent healthy tissue either when the tumors are produced by inoculation with *B. tumefaciens* or by freezing.

Growth in frozen spots of Bryophyllum leaves is correlated with the accumulation within them from the surrounding tissue of substances of the nature of catechol, which are transformed by oxidation into colored compounds.

The H^+ concentration of tumors produced both by freezing and by bacterial inoculation is consistently less than that of adjacent healthy tissue.

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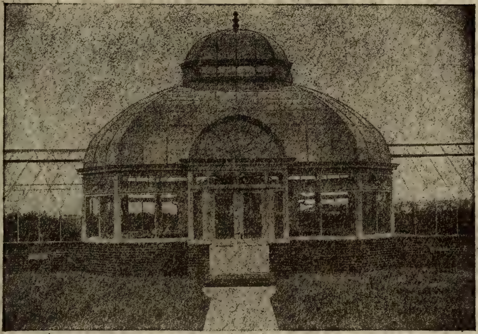

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THE FUSION OF VENTRAL CANAL CELL AND EGG IN SPHAGNUM SUBSECUNDUM

GEO. S. BRYAN

In a previous paper (1) the writer has followed in detail the development of the archegonium of *Sphagnum subsecundum* Nees. At that time the statement was made: "Usually just before fertilization the ventral canal nucleus disintegrates." However, in the early spring of 1917, while attempting to work out the details of fertilization, the interesting fact was uncovered that in the species here studied the ventral canal cell quite often does not disintegrate, but unites with the egg. It seems worth while, therefore, to report the facts in detail.

MATERIAL AND METHODS

The area from which the material came is a grassy bog of about 20 acres near Mineral Springs, Indiana, 40 miles south of Chicago. In the summer and fall of 1912 this bog contained a sufficient amount of water to prevent fires from damaging the polsters of Sphagnum which were scattered throughout the bog. The material is probably dioecious, occurring generally in well defined polsters of one sex or the other. In a few cases mixed polsters were found, but in no instances were the sex organs found together in the same head or upon the same upright branch. The well defined differences in the appearance of male and female plants when the sex organs are approaching or have reached maturity have been stated in the previous paper, but will be repeated here for clearness.

The heads of antheridial plants are decidedly globose and show variations in color from yellow-brown to red-brown and sometimes almost black. Dissection reveals antheridia most of which are apparently at or near maturity. The heads of archegonial branches are less globose and have a somewhat flattened aspect on top. There is no unusual coloring except in the conspicuous bud in the center of the head. This bud varies in color from yellow-brown to red-brown and stands out in sharp contrast to the other portions of the head. An analysis shows archegonia, some young, others almost mature, as terminal structures on short side branches very close to the apex of the main axis, the coloring matter being in the peri-

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chaetial leaves surrounding the organs. These well defined characters made field work a very simple and easy matter once they were determined.

In the latter part of November and during December, 1912, a careful survey of the bog was made, the best polsters being staked out with serially numbered stakes: one series for polsters of plants bearing only archegonia; another for those bearing only antheridia; and a third series for the areas in which the sexes were mixed together, where it was hoped to secure material for a study of fertilization. Notes on field and laboratory observations were kept, and from them the following facts are taken.

By the end of November, 1912, the weather had become very cold, the bog being frozen to the depth of several inches. Blocks of frozen plants together with the frozen mud on which they were growing were cut out with a hatchet and carried in to the laboratory for observation and study. There the blocks of plants were transferred to glass jars containing several inches of water. These jars were kept partially covered with glass plates. A dissection of the material this same evening (November 28) showed the following condition: The dehiscence of several antheridia was observed, the antherozoids being quite active, but most of the antheridia had not reached maturity. Many of the archegonia appeared to be mature, but it was difficult to find one in which the cap had burst and the pathway was open for fertilization. The ventral canal cell and the egg could be easily seen in most of the archegonia. At this time of the year they stand out as two well defined balls of cytoplasm in the center of the archegonium. These rounded protoplasts are frequently so clearly defined in the living material that they can be accurately measured with an ocular micrometer. The same is also true of the nucleus of each protoplast.

For the study of details a considerable amount of material was killed in a fluid made up as follows:

Chromic acid crystals	1 g.
Glacial acetic acid	1 cc.
Water	400 cc.

The following method was employed. Using a pair of forceps with sharp, slender points, the colored buds were snipped quickly and easily out of each head, and were either transferred immediately to the killing fluid, or, if too many sterile branches were included, the latter were cut away in water under a dissecting microscope, using needles for the purpose, and the bud was then put into the killing fluid. The numerous very short side branches bearing archegonia form a firm, compact bud which may be handled by this method without the slightest injury to the archegonia, which latter are well protected by the perichaetial leaves closely investing them.

During the period from December 1 to December 6 there were warm gentle rains. On the 6th a cold wave arrived, again freezing the bog. For the remainder of the month the weather was generally cold and dry with little snow. On December 26 a considerable amount of fresh material was brought in from the field for further study. In general there was little change to be observed in the sex organs. Very few of the antheridia had dehisced, and only occasionally was an archegonium to be found in which the cap had broken open. But in my notes there appears a fact interesting in the light of subsequent events. A number of cases were observed in which the ventral canal cell had disappeared. On this same evening a large amount of material was killed chiefly in the above mentioned fluid, using the method already described, and it is from this material that the facts here recorded were obtained. The alcohol-xylol method of dehydration and embedding in paraffin was used. The material was cut 5–6 μ in thickness on a rotary microtome. Safranin in combination with Licht Grün, and Heidenhain's iron-alum haematoxylin were used as stains.

It may be of interest to record briefly the further history of the bog and the material. Events were closely followed. The summer of 1913 was hot and very dry. The water level of the bog fell rapidly in the early summer and was never regained. In the spring of 1914 this bog and the country for several miles about were completely burned over by fires which swept the region. The Sphagnum was badly damaged but not entirely destroyed. However, subsequent fires seem to have completed the work of destruction. The writer revisited the area in the early spring of 1917 but was able to find only a few struggling plants where before there had been splendid polsters.

Historical

The appearance of the mature archegonium of Sphagnum seems first to have been described by Hofmeister (3), who represents a transverse wall as separating the ventral canal cell and the egg. The former cell is shown as smaller than the latter, this being especially true in comparing the protoplasts and the nuclei of the cells. The rounding off of the two protoplasts is clearly pictured.

A few years later Schimper (7) describes the "Keimzelle" of the fully developed archegonium as follows: "Diese sah ich bei Sphagnum immer ei- oder umgekehrt birnförmig, im letzteren Falle häufig den oberen engeren Theil von dem unteren weiteren durch eine Querwand gesondert." In his Plate 9, figure 13, he shows an archegonium with the protoplasts of egg and ventral canal cell widely separate. No wall is pictured, though he speaks of it in the text. Attention is called to the fact that the nuclei can be seen through the cells of the venter of the living archegonium. In regard to the "Keimzelle" Schimper says further: "Ich fand selbst Keimzellen, welche an beiden Enden eine Querwand zeigten (fig. 16)." Whether he observed three-celled embryos, or the result of what occasionally occurs in Sphagnum—the subsequent division of either the egg or the ventral canal cell—cannot be stated with certainty. That the three-celled structure is shown as though dissected from the archegonium would lead one to suspect the former case.

In 1872 Roze (6) draws very clearly (Pl. 1, fig. 8) in a mature archegonium of *Sphagnum cymbifolium* the rounded protoplasts of the ventral canal cell and the egg, the latter being pictured as slightly larger than the former. Roze calls the protoplasts "gonosphéries ou globules germinatifs," and refers to the two nuclei as "deux nucléoles primaires." He speaks of the persistence of the two globules (protoplasts) which he says remain up to fertilization, a condition that appears to be peculiar to Sphagnum. He finds the same characteristics in the archegonia of *Sphagnum subsecundum* and *S. acutifolium* as given above for *S. cymbifolium*.

In 1887 Waldner (8), studying the development of the sporophyte of Sphagnum, pictures (Pl. II, fig. 1), according to his explanation of the plates, a longitudinal section of a mature archegonium of *Sphagnum acutifolium* Ehrh. The egg is shown as distinctly egg-shaped, occupies the whole of the venter, and contains a large nucleus with a distinct nucleolus. A fertilized egg is also pictured (Pl. II, fig. 2), but since no adequate description is given of the details one is left in doubt as to the objects figured.

In 1897 Gayet (2) describes the egg of a mature archegonium as a large elliptical cell, being elongated in the direction of the axis of the archegonium. The nucleus is almost spherical and possesses always two nucleoli. The ventral canal cell is described and figured as biconvex.

In 1915 the writer (1, pp. 48, 49) gave the following description of events in the venter of a maturing archegonium: "The ventral canal nucleus produced by this division [*i.e.*, of the ventral cell] is peculiar, being only a trifle smaller than the egg [nucleus]; and is remarkable in that it is regularly persistent and behaves for a time just as does the egg. Not long after the division into ventral canal cell and egg the canal row begins to disintegrate (this process having a variable beginning, though quite often acropetal), but not so the ventral canal cell. Its cytoplasm begins to condense about the nucleus (the same process occurring about the egg), and soon we have in a mature archegonium the appearance of two eggs separated by a wall. Later the cytoplasm about each of these two nuclei becomes markedly condensed and rounded off and may be easily observed in the living material. Still later the wall between the two cells breaks down and the nuclei, each as the center of a ball of cytoplasm, come to lie near together in the venter of the archegonium. . . . Double venters (fig. 42), unequal division of the venter, the ventral canal nucleus larger than the egg (fig. 43), ventral canal nucleus the same size as the egg (fig. 44), and multiple eggs (fig. 45) are not of rare occurrence."

In 1916 Melin (5, pp. 300, 301) says: "Das Resultat [*i.e.*, of the division of the ventral cell] sind zwei Zellen die gewöhnlich ungefähr gleich gross sind. Manchmal kann die obere, die 'Bauchkanalzelle,' etwas kleiner aln die untere, die Eizelle, sein. Beide runden sich bald ab, und wir erhaltes zwei kugelförmige Zellen, die morphologisch so gleichartig sind, dass meiner Ansicht nach kaum ein giltiger Grund besteht, sie mit verschiedenen Namen zu bezeichnen, weshalb ich sie beide Eizellen nenne. . . . Jede der beiden Eizellen hat einen grossen ziemlich chromatinarmen Kern mit deutlichem Nucleolus; der Kern ist von ungefähr gleichförmigen Plasma umgegeben. Bald verschwindet die Zellwand zwischen ihnen, und sie liegen nun frei in der Bauchhöhle."

The fusion of the ventral canal cell and the egg has been reported but once in the Musci. In 1908 J. and W. Docters van Leeuwen-Reijnvaan (4) published a remarkable article on the sexual process and spermatogenesis in several species of Polytrichum. Briefly stated their results are as follows: In the gametophyte generation there are six chromosomes, which is also the number in the cells of the antheridium. But in the final division in the antheridium a reduction process takes place so that each antherozoid receives three chromosomes. In the archegonium the division of the ventral cell produces a ventral canal cell and an egg which are equal in size. During this division a reduction process is also said to occur so that each of these cells receives three chromosomes. The protoplasts of the ventral canal cell and the egg fuse while the neck of the archegonium is still closed. After the cap breaks open this fusion cell is fertilized by two antherozoids. In this manner the sporophytic chromosome number is restored.

In 1913 Walker (9) published the results of his study on the behavior of the egg and the ventral canal cell in *Polytrichum formosum* and *P. commune.* More than one hundred archegonial rosettes were sectioned but no case of a fusion could be found. Walker thinks the appearance of fusion of the ventral canal cell and egg reported by the van Leeuwen-Reijnvaans is due to their method of fixation.

DEVELOPMENT OF VENTRAL CANAL CELL AND EGG

The ventral cell of *Sphagnum subsecundum* generally divides late into ventral canal cell and egg. The division of cells in the neck is almost if not quite complete when this division occurs. The ventral canal cell is not only persistent but remarkably variable in size. As a very general statement one may say that this cell and its nucleus are a trifle smaller than the egg and the egg nucleus (figs. 2, 8). However, the exceptions are numerous. Often the two are identical in size both as regards the protoplasts and the nuclei (figs. 3, 4), while more rarely the ventral canal cell is larger than the egg in both of these respects (fig. 9).

Shortly after the division of the ventral cell the cells of the canal row begin to disintegrate, but this process has not as yet been found to affect the ventral canal cell. The protoplast of this cell begins to round off, the same process having begun in the egg, the wall between the two cells breaks down, and we have the appearance of two well rounded eggs which soon come more or less in contact in the venter of the archegonium (figs. 3, 8, 9). About this time there may appear, especially in the upper portions of the venter, more or less faintly staining bodies which probably take their origin from the disintegrated canal cells above. Sometimes these bodies lie close to the ventral canal cell (figs. 3, 8). A rare exception is shown in figure 4 in which a body from the neck has apparently joined itself to the protoplast of the ventral canal cell.

The Fusion of the Protoplasts

As illustrated by figures 4, 5, and 6, the protoplasts unite completely. This fusion is followed later by the union of the nuclei. Unfortunately the killing agent employed, while giving excellent morphological results and little plasmolysis, is not very satisfactory from a cytological standpoint, hence the details of chromatin behavior cannot be accurately reported. In general the chromatin of the two nuclei appears to be more or less intermingled. There is no tendency for each mass to remain distinct.

Every nucleus of *Sphagnum subsecundum* which has been observed thus far, with the exception of that of the fusion cell here described, whether it be of the gametophyte or of the sporophyte generation, is characterized by one conspicuous well-rounded nucleolus. The fertilized egg may prove an exception, since its nucleus has not been seen in a satisfactory preparation. The only other exception is to be found in this fusion nucleus. No case has yet been observed in which the two nucleoli of ventral canal cell and egg have united, though such a condition would seem perfectly possible. In all the cases so far observed the fusion occurs while the neck of the archegonium is still closed. There is, therefore, no danger of mistaking this fusion nucleus for that of a fertilized egg.

The Disintegration of the Ventral Canal Cell

In the material studied, clear cases of the disintegration of the ventral canal cell have been found a number of times. There is no doubt that the ventral canal cell frequently disintegrates; but a summary of the large number of slides studied thus far shows that the number of cases of the union of the two cells about equals the number of cases in which it is certain that the ventral canal cell has disintegrated. In a large number of cases the ventral canal cell was still persistent and was more or less in contact with the egg, as illustrated in figure 3. Two cases were found in which the nucleus of the egg appeared to be degenerating, while that of the ventral canal protoplast just above it was very clear, and sharply defined.

Discussion

It is evident from an examination of the literature quoted that the rounded appearance of the protoplasts of the mature archegonium is not due to the killing agent but is a condition which may be observed and even measured in living material. The average of a number of measurements of the diameters of living protoplasts compared with a like average of killed and stained protoplasts shows that there has been some contraction due to the killing, both in protoplasts and in nuclei, but the contraction is relatively small and could not bring about the facts which have been observed. Figures 1 and 2 show the exact amount of plasmolysis due to killing and fixing.

Furthermore, I am unable to believe that the technique employed is responsible for the fusions. Using the same methods described in this paper I killed at various times in the fall of 1913 large amounts of Sphagnum for a study of the development of the archegonium. In no cases could be found the slightest trace of injury to the sex organ at any stage of its development. No canal cells were ever observed in the venter, and only after the disintegration of the canal row did any of the contents of the neck begin to make their appearance in the venter. At a later time this disintegrated matter fills the venter with a slimy mucilaginous mass which makes the study of fertilization extremely difficult.

Still more important evidence that the technique is not responsible for the facts is that it is possible to demonstrate *stages* in the fusion of the protoplasts and the nuclei. Not only that, but on a slide from a single head appear archegonia showing the following conditions: (1) Protoplasts of the ventral canal cell and the egg not in contact. (2) Protoplasts have fused, but nuclei, while in contact, are still separate and distinct. (3) Protoplasts and nuclei have fused completely.

It seems hardly reasonable to believe that the technique could bring about the appearance of these varying stages in a single head.

Insofar as the writer is aware, the archegonium of Sphagnum is unique among the Musci in that it comes to maturity in the late fall, withstands the severity of winter, and the egg is fertilized in the early spring. It undergoes great changes in temperature in the alternate freezing and thawing of certain winters; and when snow is absent and the temperature is low it is subject not only to freezing, but no doubt to considerable drying as well. It may be that these severe external conditions furnish the stimulus which brings about the fusion of the protoplasts.

As yet I am unable to make any statement in regard to the behavior of the fusion nucleus. Whether it may develop directly into a sporophyte, whether or not it is capable of being fertilized, and whether or not this fusion is peculiar only to the species here studied—all these questions must await further work.

Summary

1. The ventral canal cell of *Sphagnum subsecundum* is regularly persistent, and variable in size.

2. The protoplasts of ventral canal cell and egg round off and, the wall between the two disintegrating, they lie near together in the venter of the archegonium.

3. In material killed in the latter part of December a number of cases of the fusion of these protoplasts have been found.

4. The fusion of the protoplasts is followed by the fusion of the nuclei.

5. Undoubted cases of the degeneration of the ventral canal cell have also been found.

6. Occasionally the egg may degenerate, while the protoplast of the ventral canal cell remains functional.

7. Further work is needed to determine how general this condition is in other species of Sphagnum, and to follow the history of the fusion nucleus.

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EXPLANATION OF PLATES

All figures were drawn at table level with the aid of a camera lucida, using Spencer ocular 10 x and 1.5 mm. oil immersion objective. Being reduced one half in reproduction, they show a magnification of approximately 1000 ×.

PLATE XIV

FIG. 1. Ventral cell of an archegonium having eight neck canal cells. Peculiarly shaped plastids in the cytoplasm.

FIG. 2. Ventral canal cell and egg still separated by a wall, showing the difference in size of the two nuclei. Contraction of the protoplasts is probably due in large part to plasmolysis.

FIG. 3. Wall has disintegrated, protoplasts have rounded off and are lying in contact in the venter. Nuclei and protoplasts practically identical in size.

FIG. 4. The two protoplasts in closer contact but outline of each distinct. Disintegrated material from canal row in contact with upper protoplast.

FIG. 5. Protoplasts have completely fused. Nuclei in contact, but each distinct.

PLATE XV

FIG. 6. The fusion of protoplasts and nuclei completed. Mucilaginous matter beginning to appear about the protoplast.

FIG. 7. The disintegration of the ventral canal cell. Only a blurred mass remains.

FIG. 8. The rounded protoplasts. That of the ventral canal cell is smaller and has a smaller nucleus than the egg.

FIG. 9. Protoplast of the ventral canal cell is larger and has a larger nucleus than the egg.

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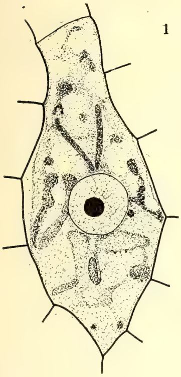

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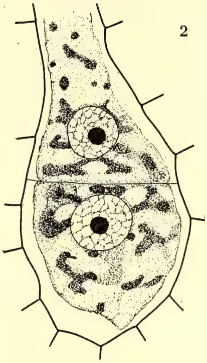

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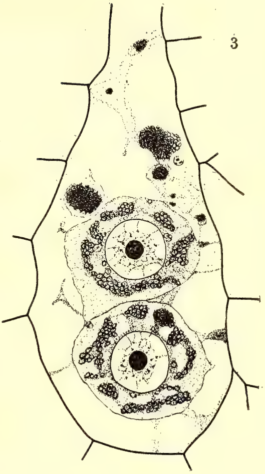

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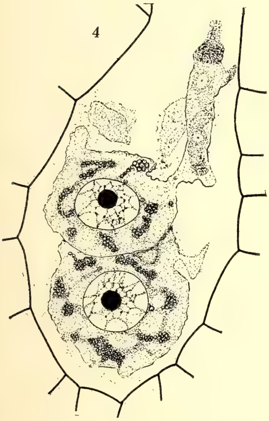

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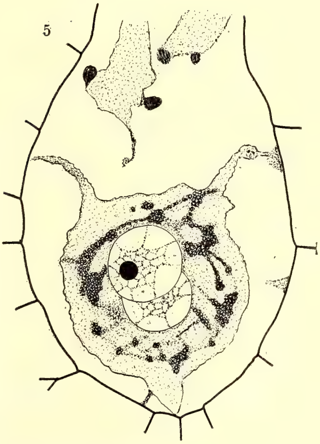

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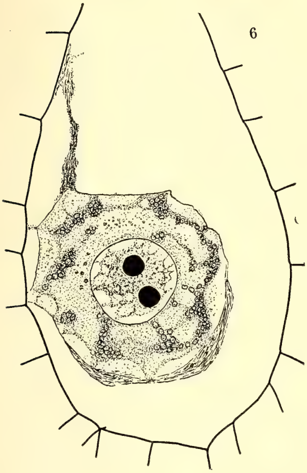
BRYAN: FUSION IN SPHAGNUM.



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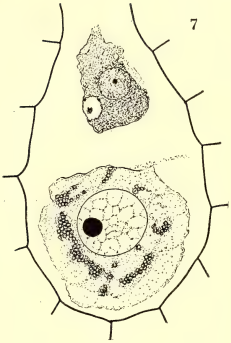

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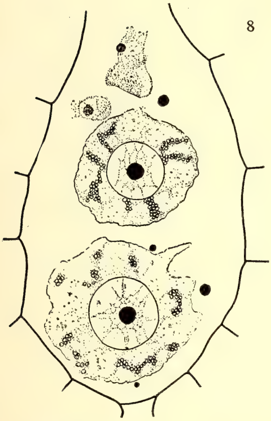

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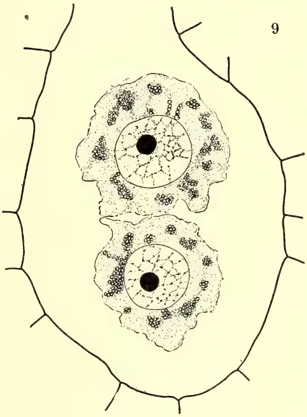

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BRYAN: FUSION IN SPHAGNUM.

THE GEOGRAPHICAL DISTRIBUTION OF NORTH DAKOTA PLANTS

O. A. Stevens

The fact that North Dakota is only partially included within the limits used by eastern and western manuals, and that its northern boundary is also that of the United States, makes the composition and distribution of its flora a question of considerable interest. The present paper is offered as a preliminary analysis of our knowledge of the subject. It is based upon the records published by Bergman (3)[1] and upon the writer's observations made during occasional visits to nearly all parts of the state in the years 1910 to 1919. The number of vascular plants found within the state as listed by Bergman is 966. This is by a conservative limitation of species. Lunell (8), using narrow specific limits, lists about 1,300. It is thus seen that the number is small as compared with that of other states. This is probably due to a comparative lack of diversity of conditions, as there is no unexplored area which might add materially to the list.

To a botanist unfamiliar with the plants west of the range of the eastern manuals, the first feature of interest is the western plants. To the writer it has always seemed that the most natural limit of the western forms is the Missouri River in the southern part of the state and a line continued across the state in the direction of its course there, which would be about longitude 100° 30′ to 101° 30′. A few of the western forms extend eastward as far as the Sheyenne River. A preliminary division into general groups according to the distribution of the plants in the state gave the following results:

I.	Eastern; not west of Red River, Devil's Lake, or Turtle Mountains	25%
II.	Eastern; reaching the Missouri River	10%
III.	Western; west of the Missouri River	11%
IV.	Western; extending east to the Sheyenne River	8%
V.	Cosmopolitan	30%
VI.	Introduced	16%

These figures are somewhat unsatisfactory, especially as to the second group, this being not well separated from the first. There is an apparent lack of records from the southern two thirds of the state from Jamestown west to the Missouri River. It is evident, however, that the western element is comparatively small. A more critical study of some of the principal families and orders with regard to their North American distribution shows the following results when grouped as in the preceding list. The number of species included in eastern (10) and Rocky Mountain (4) manuals is added to show whether the families are better represented in the east or in the west.

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[1] The nomenclature and arrangement of his work is followed in this paper.

Table 1. *North American range of North Dakota plants*

	I	II	III	IV	V	VI	Total	N. E. U. S.	R. Mt.
Ranunculaceae	2	6	4	0	16	1	29	93	89
Brassicaceae	2	1	4	1	15	23	46	102	133
Scrophulariaceae	2	1	2	1	15	3	24	115	121
Boraginaceae	0	1	3	1	5	5	15	49	67
Lamiaceae	4	0	1	0	12	4	21	119	28
Rosaceae	4	4	4	5	14	1	32	214	94
Fabaceae	6	3	7	5	20	10	51	166	201
Asteraceae	25	7	19	2	53	12	118	428	463
Liliales	8	3	5	0	12	1	29	174	62
Cyperaceae	6	2	2	1	48	0	59	333	99
Poaceae	18	0	10	3	64	21	116	378	206
Total	77	28	61	19	274	81	540		
Percent	14	5	11	4	51	15	—		

In table 1, column I includes only the strictly eastern plants; such species as are found only in the eastern part of the state but occur in the western United States or Canada are placed in column V. Columns II and IV are similarly affected. Various difficulties arise in such a compilation, chiefly through incomplete records. The statements of distribution as given in the manuals are frequently insufficient, and original or detailed records must be consulted. For general distribution chief reliance has been placed upon the statements of Rydberg (11).

The Western Plants

These are quite conspicuous in early spring on the hills, where we find such plants as: *Phlox Hoodii*, *Mertensia lanceolata*, *Viola Nuttallii*, *Orophaca caespitosa*, *Potentilla concinna*, and *Carex filifolia*. In late spring and early summer are found: *Paronychia sessiliflora*, *Eurotia lanata*, *Eriogynum flavum*, *E. multiceps*, *Chamaerhodos erecta*, *Thermopsis rhombifolia*, *Astragalus pectinatus*, *Pentstemon cristatus*, *P. angustifolius*, and *Oreocarya glomerata;* on flats or gentle slopes below the hills, *Musineon divaricatum* and *Lomatium foeniculaceum*. On bare clay buttes in the "Bad Lands" and adjacent territory, *Pachylophus caespitosus* and *Chrysothamnus graveolens* are striking plants. The northern sides of many buttes are covered on the upper parts with *Juniperus horizontalis*, this being one of the plants which is absent in the eastern part of the state although it ranges to the Atlantic coast. *Artemisia cana* is a conspicuous low shrub on the hillsides and flats below; this is the nearest approach to "sage-brush," as *A. tridentata* has been collected only at one point (Medora).

Some species are reported only from the extreme western part of the state, such as:

Delphinum bicolor
Physaria didymocarpa
Stanleya pinnata
Sarcobatus vermiculatus
Abronia micrantha
Gilia congesta
Phacelia leucophylla
Homalobus caespitosus
Kentrophyta montana
Sideranthus grindelioides
Pyrrocoma lanceolata
Stenotus armerioides
Townsendia exscapa
Calochortus Nuttallii
Juniperus communis
Juniperus scopulorum

Others, as before noted, extend eastward to the Sheyenne valley although less common eastward. Among these are: *Viola Nuttallii*, *Atriplex Suckleyana*, *Chamaerhodos erecta*, *Astragalus racemosus*, *A. bisculcatus*, *Lomatium foeniculaceum*, and *Carex filifolia*.

The western plants belong to the great plains area, ranging chiefly from Nebraska to Saskatchewan. A few, such as *Gilia congesta*, *Pentstemon angustifolius*, *Hedeoma nana*, *Kentrophyta montana*, and *Picradenia pumila* seem to have a rather limited range (Colorado, Wyoming, Utah). Many extend as far south as New Mexico (see lists on later page), while the following go west into the Pacific coast states:

Ranunculus glaberrimus
Delphinum bicolor
Arabis Holboellii
Atriplex confertifolia
Navarettia minima
Allocarya scopulorum
Phacelia leucophylla
Mertensia lanceolata
Potentilla multisecta
Chamaerhodos erecta
Lavauxia brachycarpa
Rhus trilobata
Gaertneria acanthicarpa
Erigeron pumilus
Pyrrocoma lanceolata
Artemisia cana
Artemisia dracunculoides
Artemisia tridentata
Crepis occidentalis
Calochortus Nuttallii
Eriocoma cuspidata
Poa Buckleyana

The Eastern Plants

Species found along the Red River and not extending into western North America are:

Actaea spicata
Aquilegia canadensis
Clematis virginiana
Anemone quinquefolia (rare)
Menispermum canadense
Viola conspersa (rare)
Caulophyllum thalictroides
Sanguinaria canadensis
Xanthoxylum americanum
Corylus americana
Leptandra virginica (rare)
Penthorum sedoides
Silphium perfoliatum
Aster sagittifolius
Allium tricoccum
Uvularia perfoliata
Uvularia grandiflora
Trillium cernuum
Festuca nutans
Oryzopsis melanocarpa

Others, which occur only in the eastern part of the state, range west through Canada or are found in the Rocky Mountain region of the United States. Such are:

Anemone virginiana
Impatiens biflora
Rhamnus alnifolia (rare)
Heliopsis scabra
Moldavica parviflora
Lathyrus palustris
Lathyrus venosus
Meibomia grandiflora
Petasites sagittata
Vagnera racemosa
Disporum trachycarpum
Carex Deweyana
Scolochloa festucacea
Hystrix Hystrix
Dryopteris cristata
Matteucia Struthiopteris

Eastern Limits of Prairie Plants

Many species common to the hills and higher prairie west of the Red River valley extend eastward into the prairie region of western and southwestern Minnesota. Upham (12) lists about 100 species which reach their eastern limits there. Most of these are common prairie plants in North Dakota, although absent from the lower ground of the valley or found only in certain places as upon old lake beaches and introduced with gravel on railroad grades. Such are:

Malvastrum coccineum
Asclepias speciosa
Pentstemon albidus
Castilleja sessiliflora
Meriolix serrulata
Gaura coccinea
Lepargyrea argentea
Anogra pallida
Lomatium orientale
Cymopterus acaulis
Gutierrezia Sarothrae
Chrysopsis villosa
Sideranthus spinulosus
Brauneria angustifolia
Ratibida columnaris
Gaillardia aristata
Artemisia frigida

Others, associated with these, occur much farther east, as: *Erysimum asperum*, *Eleagnus argentea*, and *Aster ptarmicoides*.

Life-Zone Distribution

As shown by the map (fig. 1), North Dakota lies almost entirely within the Transition Life Zone, the Canadian approaching closely on the northeast, the Upper Austral[2] on the south and west. Of the Transition Zone, Merriam (9) has stated that it is "as a whole, characterized by comparatively few distinctive animals and plants, but rather by the occurrence together of southern species which here find their northern limit, and northern species which here find their southern limit."

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[2] No attempt is made in this paper to distinguish between a humid and an arid portion of these zones. Sonoran as referred to on later pages is included in Upper Austral.

CANADIAN ZONE SPECIES

Upham (12) has given a list of about 75 northern species which reach their southern limit in the basin of the valley of the Red River of the North, but very few of these are known in North Dakota. The few Canadian Zone plants which occur in the state are found in the Turtle Mountains and Pembina Mountains (as far south as Milton), these being outlying points cut off from the body of the zone by a distance of 100 miles or more. The following species may be mentioned:

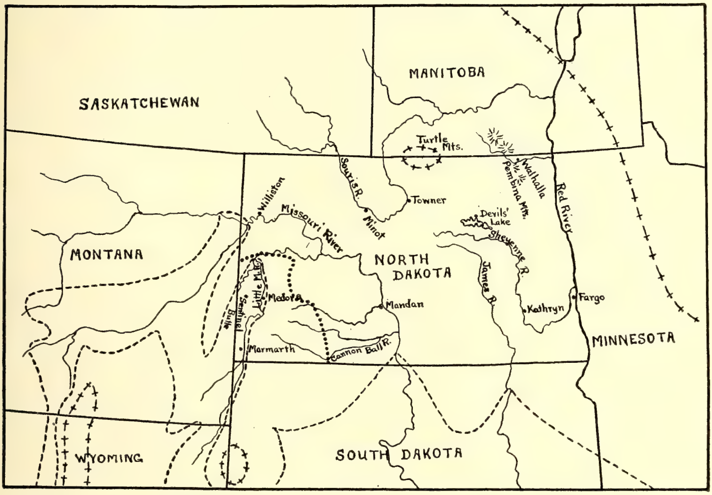
SASKATCHEWAN
MANITOBA
Turtle Mts.
Walhalla
Pembina Mts.
Red River
Souris R.
Towner
Minot
Williston
Missouri River
Devils' Lake
Sheyenne R.
NORTH DAKOTA
James R.
Kathryn
Fargo
MINNESOTA
MONTANA
Little Mo.
Sentinel Butte
Medora
Mandan
Marmarth
Cannon Ball R.
WYOMING
SOUTH DAKOTA

FIG. 1. Sketch map of North Dakota and adjoining states showing localities mentioned and life-zone boundaries as given by United States Biological Survey.[3] + + + lower limit of Canadian Zone;—upper limit of Upper Austral Zone (Transition Zone between); . . . southern limit of glaciated area in North Dakota.

Anemone Hudsoniana
Corylus rostrata
Geranium Bicknellii
Polygala Senega
Pyrola asarifolia
Arctostaphylos Uva-ursi
Oxytropis deflexus
Mitella nuda
Ribes triste
Circaea alpina
Lepargyrea canadensis
Cornus canadensis

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[3] According to the fourth provisional map (1910). Mr. Vernon Bailey, who has read the manuscript of this paper, writes that in a report on the mammals of North Dakota which he has about completed, he has extended the Upper Austral limit to the mouth of the Cannon Ball River and a little way up that river; also a little farther in the western valleys.

Erigeron lonchophyllus	*Corallorhiza multiflora*
Achillea multiflora	*Cinna latifolia*
Senecio eremophilus	*Avena Torreyi*

It is noteworthy that most of these are plants of wide distribution in North America except Oxytropis, Avena, and the three composites, which are western. Of the foregoing, Anemone, Pyrola, Avena, and Erigeron are recorded from Devil's Lake, the last-named only from there. Another touch of Canadian Zone plants is evident in deep ravines at Kathryn where *Pyrola secunda*, *Corylus rostrata*, and *Circaea alpina* have been found. *Oxytropis splendens* occurs throughout the northern part of the state, but not over 50 miles southward so far as known.

Upper Austral Species

This zone reaches the borders of the state at three points, *viz.*: the valley of the Missouri River on the south and northwest and that of the Little Missouri on the southwest. *Pentstemon grandiflorus* is common along the Missouri River at least as far as Mandan and up the Cannon Ball for some distance; *Talinum parviflorum*, *Eriogynum annuum*, *Specularia perfoliata*, *Erigeron caespitosus*, *Leptilon divaricatum*, *Munroa squarrosa*, *Diplachne fascicularis*, and *Pellaea atropurpurea* are known only from the lower Missouri; *Atriplex confertifolia*, *Sarcobutus vermiculatus*, *Abronia micrantha*, and *Astragalus gracilis* (Marmarth, Stevens in 1918), only from the Little Missouri. *Clematis ligusticifolia*, *Psoralea lanceolata*, *Aster pauciflorus*, and *Oryzopsis micrantha* are known from the upper Missouri as well. (For further notes on austral species see under "Comparison with the Flora of New Mexico.")

A considerable representation of austral forms is found also in the southeastern part of the state where the Sheyenne River turns northward. From here only is known *Bouteloua hirsuta;* other noteworthy species are: *Pentstemon grandiflorus*, *Mimulus Geyeri*, *Bacopa rotundifolia*, *Verbena stricta*, *Lythrum alatum*, and *Cenchrus tribuloides*. To this list Mr. Reynold Shunk added in 1917: *Euonymus atropurpureus*, *Sicyos angulatus*, and *Cyperus diandrus*.

Sand Dune Species

Two small areas of drifting sand occur in the state, one at the locality last mentioned, the other near Towner. From these only are recorded: *Euphorbia Geyeri*, *Petalostemon villosum*, *Lygodesmia rostrata*, *Cyperus Schweinitzii*, and *Redfieldia flexuosa*. From near Towner we have *Cycloloma atriplicifolium* and *Andropogon Hallii* which do not elsewhere in the state occur so far north.

Records of Special Interest

At Fargo there is a slight intermingling of northern and southern species. *Lappula americana*, which occurs there, must reach nearly its southeastern limits in that region; it is reported as far as Nebraska (11) and Iowa (10) but from Minnesota for the first time in 1901 (13) from farther north. On the Minnesota side near Fargo is a colony of *Petasites sagittata* which seems to be the most southeastern point known for that species. *Pyrola elliptica* and *Viola conspersa* are found in the same piece of aspen woods. *Ilysanthes dubia*, an austral species, has been found in quantity at Wild Rice which is ten miles farther south. *Celtis occidentalis*, which extends irregularly farther north, is frequent at Fargo.

At Walhalla there is a typical butte, said by Willard (14) to be perhaps the easternmost in the United States. Upon this grow a number of plants which are otherwise found only in the western part of the state, such as *Eriogynum flavum*, *Artemisia cana*, and *Juniperus horizontalis*. In the woods of the river near by occur a number of woodland plants which are known from no other locality in the state: *Mitella nuda*, *Rhamnus alnifolia*, and *Asarum acuminatum*, which are purely eastern species; also *Cardamine pennsylvanica*, *Petasites sagittata*, *Leptorchis Loeselii*, *Carex leptalea*, and *Cinna latifolia*, which also occur in western North America.

From Sentinel Butte and Medora only, is recorded *Dasiphora fruticosa*. This is a Canadian to Arctic Zone plant of wide distribution and these stations are perhaps to be regarded as cut off from the Black Hills region, but it seems strange to find this plant where there is a prominent representation of austral forms. Likewise, *Juniperus horizontalis*, which is mainly a Canadian Zone species, is common on the buttes and hills along the Missouri and westward.

The distribution of the two species of lupine (*Lupinus argenteus* and *L. pusillus*) extends, as far as known, about to the southern limit of the glaciated area as indicated by Leonard (7).

INTRODUCED SPECIES

Of the introduced species, the abundance of Brassicaceae and Chenopodiaceae (including such recent introductions as *Erucastrum Pollichii*, *Camelina dentata*, and *Axyris amaranthoides*), and the scarcity of Polygonaceae (*Rumex* spp. and *Persicaria* forms) and of Euphorbiaceae are noteworthy. Of Compositae, *Carduus arvensis* and *Sonchus arvensis* are by far the most important.

The observations made by Upham (12) thirty years ago are interesting in comparison with present conditions. He notes of *Thlaspi arvense*: "long established and very abundant in the vicinity of Winnipeg, recently spreading into Minnesota and North Dakota." Of *Taraxacum officinale:* "frequent along roadsides, in pastures, etc., about Winnipeg and plentiful at the west end of the main street in St. Vincent; generally rare or absent throughout the Red River Valley." Both of these plants have since become particularly abundant in the valley. He also notes the absence of *Ambrosia trifida* (native weed) from the district about Langdon where it is absent still. *Sonchus arvensis* he does not mention, it having been introduced in Manitoba a few years later and being now common through the valley and spreading farther.

Neslia paniculata and *Camelina sativa* seem to be restricted mainly to the northeastern two or three counties, *Camelina dentata* being widely distributed in flax. Certain Poaceae are especially abundant and characteristic: *Avena fatua*, *Hordeum jubatum*, and *Agropyron repens*. *Chaetochloa glauca*, *C. viridis*, *Echinochloa crus-galli*, and *Panicum capillare* are also very abundant but perhaps less characteristic. *Syntherisma sanguinalis* is repeatedly introduced in southern-grown millet seed but has not been collected in the state even in adventive condition. *Plantago lanceolata* has been collected a few times but apparently does not become established.

Comparison with the Biological Survey Reports

Considerable difficulty has been found in trying to correlate the distributional data with those of previous publications dealing with life zones. The Rocky Mountain region has been described in considerable detail, but in the plains region, as noted by Cary (6), the zone limits are less well marked. Trees have been used to a great extent in indicating the boundary lines of the zones, and North Dakota is especially lacking in trees.

Pinus scopulorum is mentioned by Cary (5) as "the characteristic Transition tree," but in North Dakota it is found only on the hills in the extreme southwestern part of the state where the Austral Zone invades the river valleys. *Populus tremuloides*, according to the same author, "is perhaps the best characterizing tree of the Canadian Zone" in Wyoming (6). He also mentions it as "restricted to the Canadian Zone" in Colorado, while Bailey (2) lists it for the same in New Mexico. Apparently it is most abundant in this zone east of the mountains, but it is common through the Transition Zone and extends half way through the Upper Austral in the Mississippi Valley region. *Quercus macrocarpa* is noted as growing in the Transition Zone in Wyoming (6), but in the Mississippi Valley region where it is more abundant it extends from the Canadian southward well into the Lower Austral.

Of the trees and shrubs listed by Cary as Upper Sonoran in Wyoming, at least *Salix amygdaloides*, *S. fluviatilis*, *Atriplex argentea*, *Amorpha nana*, and *Gutierrezia Sarothrae* are common in North Dakota Transition, while the same may be said of fully one fourth of the "herbaceous plants." In the same paper *Psoralea argophylla* is listed as Transition, although it is common on the plains through Upper Austral; *Actaea rubra*, *Sieversia ciliata*, and *Heracleum lanatum*, listed as Canadian, are common in North Dakota Transition.

Comparison with the Flora of New Mexico

The fact that Wooton and Standley (15) have indicated the zonal distribution for practically all the species found in New Mexico has made it desirable to examine the distribution of such North Dakota plants as occur there. A tabulation shows that 418 of the 966 North Dakota plants (43.3%) are recorded in New Mexico. Of these the distribution in the latter state is:

Introduced	68	Transition	77
Aquatic or not stated	42	Transition to Canadian	15
Lower Sonoran	3	Transition to Hudsonian, etc.	3
Upper Sonoran	126	Canadian or higher zones	11
Sonoran to Transition	73		

(Of those listed here as Upper Sonoran, many range also in Lower Sonoran, but only their northward distribution is considered here.)

Fourteen of the Sonoran species have been mentioned in a preceding paragraph as entering North Dakota at one or more of the three points where that zone touches the state. About three fourths of the number extend into Transition, and these it seems convenient to divide into two groups. The following seem to extend only part way into Transition (occasional over the area southwest of the Missouri or in the southern part of the state east of that river):

Myosurus minimus
Polanisia trachysperma
Euphorbia serpens
Euphorbia glyptosperma
Euphorbia Geyeri
Cycloloma atriplicifolium
Eurotia lanata
Parietaria pennsylvanica
Plantago Purshii
Acerates viridiflora
Solanum rostratum
Hedeoma nana
Lupinus pusillus
Parosela enneandra
Strophostyles pauciflora
Anogra albicaulis
Opuntia fragilis
Opuntia polycantha
Iva axillaris
Gaertneria acanthicarpa
Chrysothamnus graveolens
Stenotus armerioides
Townsendia exscapa
Hymenopappus filifolius
Bahia oppositifolia
Tetraneuris acaulis
Helianthus petiolaris
Tradescantia occidentalis
Yucca glauca
Cyperus inflexus
Cyperus Schweinitzii
Scirpus americanus
Carex filifolia
Andropogon Hallii
Sorghastrum avenaceum
Sporobolus cryptandrus
Sporobolus asperifolius
Bulbilis dactyloides

The following species of the Sonoran list extend through the greater part of Transition in North Dakota, most of them being common plants over a large part of the state:

Erysimum asperum
Malvastrum coccineum
Viola pedatifida
Atriplex hastata
Atriplex argentea
Amaranthus blitoides
Allionia hirsuta
Salix amygdaloides
Salix interior
Rumex persicarioides
Polygonum lapathifolium
Androsace occidentalis
Solanum triflorum
Castilleja sessiliflora
Heliotropum spathulatum
Lithospermum linearifolium
Onosmodium occidentale
Verbena hastata
Lycopus lucidus
Lycopus americanus
Amorpha canescens
Petalostemon purpureum
Astragalus crassicarpus
Astragalus missouriensis
Glycyrrhiza lepidota
Vicia sparsiflora
Gaura coccinea
Meriolix serrulata
Onagra pallida
Lomatium orientale
Lacinaria punctata
Grindelia squarrosa
Sideranthus spinulosus
Aster oblongifolius
Aster multiflorus
Coreopsis tinctoria
Bidens frondosa
Artemisia frigida
Lactuca ludoviciana
Lygodesmia juncea
Sagittaria arifolia
Typha latifolia
Scirpus campestris
Scirpus atrovirens
Eleocharis Engelmannii
Eleocharis palustris
Carex gravida
Andropogon furcatus
Panicum virgatum
Stipa comata
Stipa spartea
Calamovilfa longifolia
Schedonnardus paniculatus
Phragmites Phragmites
Distichlis spicata
Puccinellia airoides
Elymus Macounii

Of the New Mexican species recorded for Transition the following are common also in the Upper Austral southward from North Dakota (based chiefly upon the writer's knowledge of them in eastern Kansas):

Anemone canadensis
Anemone cylindrica
Oxalis violacea
Urtica gracilis
Steironema ciliatum
Apocynum hypericifolium
Gratiola virginiana
Ribes aureum
Epilobium adenocaulon
Epilobium lineare
Sanicula marylandica
Washingtonia longistylis
Galium triflorum
Solidago missouriensis
Solidago serotina
Heliopsis scabra
Carex lanuginosa
Phalaris arundinacea
Muhlenbergia mexicana
Agrostis hyemalis
Sphenopholis obtusata
Koeleria cristata

Poa pratensis
Panicularia nervata
Filix fragilis
Equisetum arvense

The remaining few species to be mentioned may be tabulated as follows:

Species	in New Mexico	in North Dakota
Euphorbia serpens	Lower Sonoran	Enters Transition slightly
Cyperus erythrorhizos	" "	" " "
Polygonum emersum	" "	Common through Transition
Disporum trachycarpum	Transition to Canadian	Scarcely below Canadian
Carex aurea	" " "	Somewhat into Transition
Calamagrostis canadensis	" " "	" " "
Pyrola secunda	" " "	In Transition locally
Pyrola elliptica	" " "	" " "
Rumex occidentalis	" " "	Through Transition generally
Chamaenerion angustifolium	" " "	" " "
Heracleum lanatum	" " "	" " "
Androsace occidentalis	" " "	" " "
Triglochin palustris	" " "	Reaches Austral limits
Juncus longistylis	" " "	" " "
Calochortus Nuttallii	" " "	Near Austral limit only
Rudbeckia laciniata	" " "	Through Transition and Austral farther south
Vagnera stellata	" " "	Through Transition and Austral farther south
Agrostis hyemalis	" " "	Through Transition and Austral farther south
Dasiphora fruticosa	Transition to Arctic Alpine	(Noted under "Records of Special Interest")
Viola adunca	Transition to Hudsonian	Agrees so far as it occurs
Arctostaphylos Uva-ursi	" " "	Mainly Canadian
Oxytropis deflexus	Canadian	Canadian
Circaea alpina	"	"
Corallorhiza multiflora	"	"
Alsine longifolia	"	Well through Transition
Panicularia borealis	"	" " "
Agropyron Richardsonii	"	" " "
Agropyron caninum	Hudsonian	" " "
Pyrola asarifolia	Canadian to Hudsonian	Canadian
Oryzopsis asperifolia	" " "	"
Zygadenus elegans	" " "	Well through Transition
Juniperus siberica	" " "	Only in southwest at Austral limit
Hierochloe odorata	Hudsonian to Arctic Alpine	Through Transition in eastern N. D.

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LONGEVITY OF THE SEEDS OF CEREALS, CLOVERS, AND TIMOTHY

H. B. Sifton

This paper is the result of an investigation begun at the Seed Branch, Ottawa, in 1900. The object was to determine the longevity of some of our common crop seeds when kept under favorable and uniform storage conditions. Samples of the cereal crops of 1900, 1901, and 1902, and of the clovers and timothy of 1902 and 1903, were collected. By working with samples of all the standard varieties of the time, collected in successive years from the same farmers, in representative parts of Canada, it was believed that valuable generalizations could be drawn. Four hundred and thirty-eight samples in all were collected. Some of these proved to be too small, and were completely used up before the life of the seed was ended. A sufficient number remain, however, to allow reliable averages to be obtained.

The seeds were stored in cotton sacs or manila envelopes which were kept at ordinary room temperature in a galvanized iron chest with a lid. Once a year, in late summer, they were tested for germination. The records thus permit a comparison of their viability after various lengths of time in storage. The results of tests are recorded in the tables, to which reference will be made as each species is under consideration.

The first results were recorded in 1903, so that in the case of seeds collected in 1900 and 1901 we do not know the percentage germination for the first years. In isolated cases, results of later tests are not available as is shown by gaps in the tables. In calculating averages they have in each case been recorded to the nearest whole number.

Wheat

Of spring wheat, forty-seven samples from the crop of 1900, sixty from that of 1901, and sixty-three from that of 1902 were collected. They represent fourteen varieties and were obtained directly from farmers and from the same stock in successive years, grown in representative localities in all the provinces of Canada.

The curve in figure 1 is drawn from the average germination of all these samples for each year of their age. Practically all the kernels retain their vitality for the first five years. Then the weaker ones begin to die, and the curve gradually becomes steeper. More than 75 percent of the seeds lose their vitality between the ages of eleven and fifteen years, and about one half of these die in their 13th year. After the 15th year, the curve begins

TABLE I. *Germination of spring wheat at different ages*

Province	No. of Samples	Year of Growth	1 Yr. Old	2 Yrs. Old	3 Yrs. Old	4 Yrs. Old	5 Yrs. Old	6 Yrs. Old	7 Yrs. Old	8 Yrs. Old	9 Yrs. Old	10 Yrs. Old	11 Yrs. Old	12 Yrs. Old	13 Yrs. Old	14 Yrs. Old	15 Yrs. Old	16 Yrs. Old	17 Yrs. Old	18 Yrs. Old	19 Yrs. Old
Nova Scotia	8	1901		100	99		100	100	100	99											
	8	1902	99	99		100	84	99	99	99	99	99	96	70	42	40	9	3	1		
Prince Edward Island	7	1900			99	100	98	99	99	98	96	92	87	84	56	26	1	1	0	0	
	7	1901		100	100		99	99	99	98	96	94	93	56	18	1	1	0			
	8	1902	99	100		100	99	100	99	99	100	100	96	88	59	54	22	13	8		
New Brunswick	8	1900			99	99	99	99	98	98	97	96	93	93	67	37	15	6	2	1	0
	8	1901		100	99		99	99	100	98											
	8	1902	99	100		100	100	99	98	99	99	99	95	74		35	10	3	2		
Quebec	9	1900			99	98	98	94	88	81	75	71	60	53	34	11	7	3	0	0	0
	8	1901		99	99		99	99	99	98	98										
	7	1902	97	98		98	97	98	96	94	93	91	74	57	39	46	26	15	11		
Ontario	6	1900			100	100	99	100	99	98	97	94	93	91	69	38	13	4	1	2	0
	8	1901		99	100		99	99	99	97	96	96	97	55	26	5	3	1	0	0	
	8	1902	99	100		100	99	99	99	99	99	99	93	68	33	30	12	30	5		
Manitoba	8	1900			100	100	100	100	99	99	97	96	91	83	53	19	2	2	0	1	0
	8	1901		98	98		98	97	96	92	92	92	90	66	32	7	4	1	0	0	
	8	1902	100	100		100	100	100	100	99	99	99	96	78	44	36	12	7	1		
Northwest Territories	8	1900			100	99	98	95	91	88	80	72	60	54	23	7	0	1	0	0	0
	8	1901		98	96		95	90	88	83	80	75	75	51	32	32	7	1	1	0	
	8	1902	99	97		99	99	98	97	97	95	95	75	50	21	18	3	1	1		
British Columbia	1	1900			100	100	100	99	94	98	88	82	67	72	23	11	1	1	0	0	0
	5	1901		100	99		100	100	100	98											
	8	1902	100	100		100	100	100	97	99	99	99	92	78	54	31	21	9			
Average			99	99	99	99	99	98	97	96	94	92	87	69	37	28	11	5	2	0	0

to flatten again. Its direction in the seventeenth year is practically parallel to that in the tenth, and in the eighteenth and nineteenth years its direction corresponds with that in the fifth and sixth years.

When an average sample of wheat is stored for a long period, this investigation shows that its depreciation is divided into three more or less distinct periods. During the first few years (ten or eleven on the average for our Canadian samples), the weak grains gradually die. After this comes the period when the seeds of average vitality (forming the bulk of the sample) die very rapidly. A few seeds, very tenacious of life, are still left, and slowly lose their vitality during the final period of about three years.

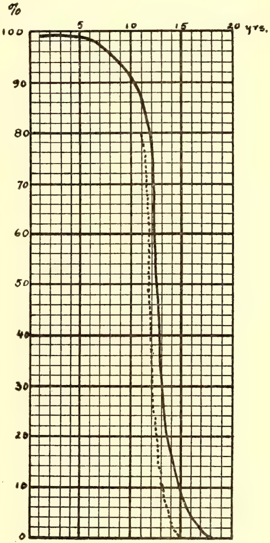

Fig. 1. Longevity curve for spring wheat.

It would be interesting for some one who has proper facilities at his disposal to raise plants from the seeds of these three divisions separately, and determine whether there are separate genotypes with different powers of resistance and whether their hardiness is transmitted to the plants which they produce and to their descendants. The work of Crocker and Groves (Proceedings of the National Academy of Sciences, March, 1915) tends to show that the loss of viability is due to the coagulation of the cell proteins, and if this is so it would be unnecessary to wait for years for the seeds to die at ordinary temperatures. Their vitality could be destroyed by carefully regulated heat after the manner used by these investigators.

The results so far considered have to do with total germination percentages as determined by a ten-day test in a standard germinator at alternating temperatures of 20° C. to 30° C. Unfortunately complete records of the preliminary four-day tests, which are believed to give an indication of the energy of germination, are not available. Records of these were kept after the tenth year, however, and are recorded on the graph by means of the dotted curve, which follows a course practically parallel to that of the main curve. During the second period of depreciation the preliminary count is from 6.5 to 8 percent lower than the final count. The energy of germination of a considerable portion of the seeds which are to die during the next year has been weakened. During the third period there is a smaller absolute difference between the two results, but a greater comparative difference; *i.e.*, of the seeds left alive after the end of the second period, the greater proportion have lost most of their energy of germination.

During the first period, if records had been preserved, there is no doubt that we should have found the preliminary and final counts approximating more closely. Although Crocker and Groves, in their work mentioned above, took no account of the depreciation of the seeds after 25 percent of them had died, their results are in agreement with those here obtained. They observe that age lowers the percentage germination and increases the time required for sprouting.

Deviations from the average curve shown in figure 1 are found in some individual cases. An attempt was made to connect these with the meteorological conditions of the year and place of growth. No satisfactory results were obtained, as meteorological statistics for the exact localities where samples were gathered had not been taken.

The variations generally are of two kinds. In very strong samples the tendency is for the first period to be longer, the second shorter, and the division between them much sharper than in average cases. For example, of the Nova Scotia crop of 1902, of which eight samples were investigated (see table 1) 96 percent germinated when twelve years old. After another year only 70 percent were alive, and in three more years the germination was reduced to 9 percent—very slightly above the average for that age. In weaker samples the tendency is toward a flattening of the curve. The Prince Edward Island crop of 1900 and the Ontario crops of 1900 and 1901 are examples of this. Extreme examples are to be seen in the Northwest Territories crop of 1901 and in the Quebec crop of 1900.

No marked difference in longevity has been observed between different varieties.

OATS

One hundred and seventy-nine samples of oats were used—fifty-two from the 1900 crop, sixty-four from that of 1901, and 63 from that of 1902. They include thirty varieties.

Their longevity is much greater than that of wheat, possibly owing to the protection of the hulls. 41 percent of the nineteen-year-old kernels are still alive. The longevity curve for the oats differs from that of wheat in two respects. The first period is longer, and the drop in the second period is not nearly so steep, *i.e.*, the kernels live longer and there are more variations in their span of life. In the year 1900, conditions seem to have been less favorable for oats than the normal conditions. The vitality of samples gathered in this year falls off much more rapidly than is the case for 1901 and 1902, and this circumstance makes the second division of the curve in figure 2 considerably steeper than it otherwise would have been. In some cases the 1900 oats start out with a high germination, but in a few years they fall below the crops of other years. Compare for example the Manitoba crops of 1900 and 1902, as recorded in table 2. During the growing season of 1900 the temperature in general was higher than in 1902 when the strongest oats were produced. Slower growth due to low temperature may

TABLE 2. *Germination of oats at different ages*

Province	Samples Used	Year of Growth	1 Yr. Old	2 Yrs, Old	3 Yrs. Old	4 Yrs. Old	5 Yrs. Old	6 Yrs. Old	7 Yrs. Old	8 Yrs. Old	9 Yrs. Old	10 Yrs. Old	11 Yrs. Old	12 Yrs. Old	13 Yrs. Old	14 Yrs. Old	15 Yrs. Old	16 Yrs. Old	17 Yrs. Old	18 Yrs. Old	19 Yrs. Old
Nova Scotia	8	1900			98	97	98	99	97	97	97	95	96	93	94	77	69	56	42	27	27
	8	1901		99	99		98	99	99	99											
	8	1902	97	98		98	98	97	100	97	99	98	99	97	98	98	95	92	91		
Prince Edward Island	9	1900			98	98	99	99	99	98	99	97	99	97	96	95	86	75	58	48	42
	8	1901		99	97		100	99	99	99	98	99	98	98	96	94	89	84	80	77	
	8	1902	97	98		100	99	99	100	98	100	99	99	98	99	99	87	89	93		
New Brunswick	6	1900			99	99		100	99	99	99	98	99	97	97	90	83	73	61	55	49
	8	1901		98	99		100	99	99	99											
	8	1902	95	98		99	99	99	99	95	99	99	97	98	95	95	91	85	86		
Quebec	8	1900			93	96		97	96	97	96	94	96	92	92	87	78	64	55	48	43
	8	1901		98	99		100	98	99	99	98	97	98	96	92	83	80	64	65	64	
	8	1902	97	99		98	97	99	98	99	99	98	98	97	91	92	88	86	86		
Ontario	7	1900			97	97	98	99	97	98	98	95	96	93	95	91	85	77	59	52	50
	8	1901		98	98		99	97	99	98	97	97	99	95	86	81	80	73	62	60	
	8	1902	97	99		98	97	100	98	97	98	98	97	97	96	97	92	84	79		
Manitoba	4	1900			98	99	99	98	93	99	99	97	97	96	95	90	96	67	59	44	42
	8	1901		99	97		99	99	100	99	98	98	98	98	96	93	89	81	79	78	
	7	1902	90	91		92	93	94	92	90	93	93	91	89	85	90	83	77	76		
Northwest Territories	4	1900			75	76		80	82	80	80	78	78	76	74	67	57	48	37	34	35
	8	1901		93	91		91	91	89	84	80	80	81	72	72	58	57	50	45	50	
	8	1902	96	96		97	97	98	96	93	98	95	92	90	84	83	72	68	58		
British Columbia	7	1900			95	95		97	96	96	95	95	98	90	93	86	81	64	53	46	41
	8	1901		99	97		100	100	99	99											
	8	1902	93	97		98	98	97	98	96	99	99	96	95	92	95	90	90	88		
Average			95	97	96	97	98	98	97	96	97	96	96	93	91	88	83	74	68	53	41

have produced hardiness, or it is possible that owing to the warmer weather of 1900 more seed was produced by weak strains of plants.

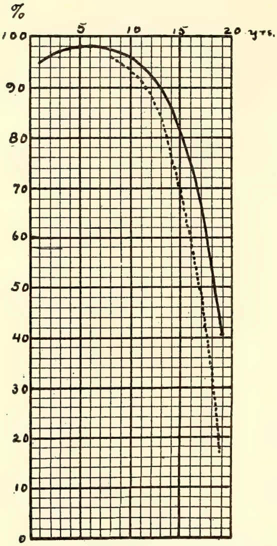

FIG. 2. Longevity curve for oats.

The sample obtained from the Northwest Territories in 1900 germinated in very low proportions even when fresh. This is due no doubt to the fact that a cold wave passed over that part of the country in August and in most parts the temperature was several degrees below freezing at some time during the ripening period.

In the majority of cases there is a slight rise in the germination of oats during the first four or five years of storage. While it has been known for a long time that their germination improved during the winter after harvesting, I have seen no account of the continuation of this improvement beyond the first year. The rise is not found in every case. As a rule it is more pronounced in the poorer samples. There is one exception in the Northwest Territories crop for 1901, which germinated in rather low proportions after two years, and from then on showed a gradual decrease.

The difference between the final germination (in 10 days) and the preliminary count (in 4 days) increases with increasing age.

The tests were carried on at temperatures alternating between 30° C. in the daytime and 20° C. at night. Later experience has proved that these temperatures are not as satisfactory for northern grown oats as temperatures somewhat lower.

TIMOTHY

Twenty-five samples of timothy were used for the investigation. Twelve were raised in 1902 and thirteen in 1903.

The curve for these is shown in figure 3. The seed begins to depreciate in value at once, and the life of the strongest kernels is comparatively short. The three periods of depreciation are not marked off so sharply from each other as in the case of hardier seeds. The germination begins to fall off rapidly after the seventh year, when it is 84 percent. By the twelfth year it is reduced to 11.5 percent, and after that the curve flattens again until at seventeen years of age the seed is practically all dead.

No records of preliminary counts were kept until the tenth year, when only 54 percent of the seed was left alive, and such as are available are therefore of little or no value.

TABLE 3. *Germination of timothy at different ages*

No. of Samples	Year of Growth	1 Yr. Old	2 Yrs. Old	3 Yrs. Old	4 Yrs. Old	5 Yrs. Old	6 Yrs. Old	7 Yrs. Old	8 Yrs. Old	9 Yrs. Old	10 Yrs. Old	11 Yrs. Old	12 Yrs. Old	13 Yrs. Old	14 Yrs. Old	15 Yrs. Old	16 Yrs. Old	17 Yrs. Old
12.....	1902	97	96	91	96	90	87	83	74	63	58	24	7	2	3	1	1	0
13.....	1903		93	94	92	90	86	85	81	72	52	23	13	14	5	3	2	
Average....		97	94	93	94	90	86	84	77	68	54	23	10	8	4	2	1	0

TABLE 4. *Germination of alsike at different ages*

No. of Samples	Year	1 Yr. Old	2 Yrs. Old	3 Yrs. Old	4 Yrs. Old	5 Yrs. Old	6 Yrs. Old	7 Yrs. Old	8 Yrs. Old	9 Yrs. Old	10 Yrs. Old	11 Yrs. Old	12 Yrs. Old	13 Yrs. Old	14 Yrs. Old	15 Yrs. Old	16 Yrs. Old	17 Yrs. Old
12.....	1902	93	90	85	84	79	70	67	54	49	45	34	14	18	17	20	19	19
12.....	1903	93	92	91	87	81	77	72	65	63	45	25	28	29	30	24	30	
Average....		93	91	89	85	80	73	70	60	56	45	29	20	23	24	22	24	19

TABLE 5. *Germination of red clover at different ages*

No. of Samples	Year of Growth	1 Yr. Old	2 Yrs. Old	3 Yrs. Old	4 Yrs. Old	5 Yrs. Old	6 Yrs. Old	7 Yrs. Old	8 Yrs. Old	9 Yrs. Old	10 Yrs. Old	11 Yrs. Old	12 Yrs. Old	13 Yrs. Old	14 Yrs. Old	15 Yrs. Old	16 Yrs. Old	17 Yrs. Old
12.....	1902	97	93	86	80	77	65	61	57	48	44	27	7	15	11	14	8	9
12.....	1903	96	96	92	86	75	68	68	59	59	43	17	21	29	28	16	23	
Average....		96	94	90	83	76	66	65	58	54	44	22	14	23	18	15	17	9

Alsike and Red Clover

Twelve samples of each of these from the 1902 crop and twelve raised in 1903 were used, and the tests were carried on at 18° to 20° C. The curves are shown in figures 4 and 5. The interesting thing about them is their regular decline from the first, making each curve, as far as it has yet gone, approach a straight line much more closely than in the case of any other crop studied. Both clovers have a larger proportion of long-lived seeds (over 15 years) than wheat, but from the standpoint of a practical seedsman their longevity is not nearly so great. After eleven years, wheat on the average still germinates to the extent of more than 85 percent, but eleven-year-old alsike or red clover seed germinates less than 40 percent. (This result is calculated from the curve. The actual germination obtained in the laboratory on eleven- and twelve-year-old samples was much less, but the results of later and earlier tests make it evident that the percentages obtained in these two years were not correct.)

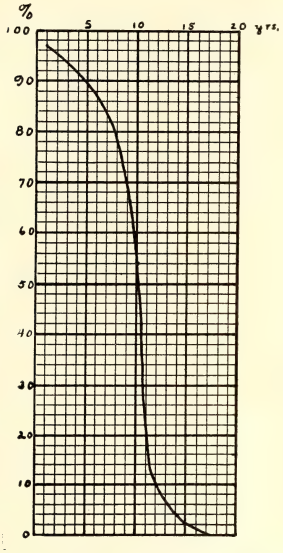

FIG. 3. Longevity curve for timothy.

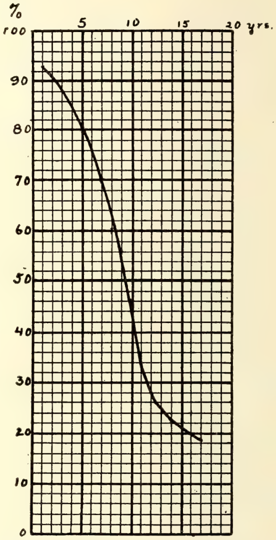

FIG. 4. Longevity curve for alsike.

The natural expectation would be that the curves for the clovers would be almost horizontal at the first owing to the gradual softening of "hard seeds" with age. This, in the course of an experiment not yet ready for publication, has been proved to be true in alfalfa. The samples contained a considerable proportion of "hard seeds," and for a few years the germination percentage increased as they became permeable.

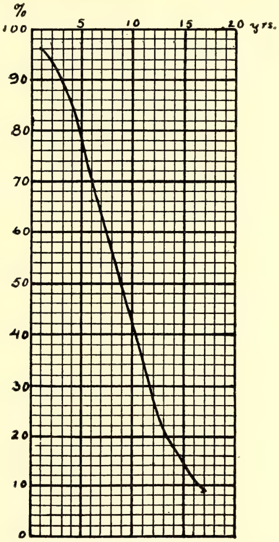

FIG. 5. Longevity curve for red clover.

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OTTAWA, CANADA

ON THE ANATOMY OF CHENOPODIUM ALBUM L.

Ernst F. Artschwager

Introduction

The Chenopodiaceae and related families exhibit a most striking anomalous structure of the stem in that the annual secondary thickenings contain several circles of collateral vascular bundles of limited development which are embedded in lignified so-called "conjunctive tissue."

Gheorghieff (1) in a series of publications gives a detailed review of the early literature on this subject. His own contributions, furthermore, comprise the most comprehensive study of the anatomy of the Chenopodiaceae. He finds that the plants which he examined show greatly varied forms, transitional in structure to many of the Centrospermae.

Sanio (2) in 1863 gives the most complete ontogenetic study of members of the Chenopodiaceae. He attributes the anomalous structure of the stem to the activity of a periodically acting cambium which produces collateral vascular bundles and "conjunctive tissue." At the conclusion of his work Sanio draws a comparison between the anomalous stem structure of the Chenopodiaceae and the stem structure of those monocotyledons which are characterized by growth in thickness.

In his "Comparative Anatomy of the Phanerogams and Ferns," De Bary (3) develops a theory to account for the diverse forms of anomalous growth of the vascular tissue of Chenopodiaceae and related families. He makes four general classes. In the plants of the first group, an extrafascicular cambium appears around the primary ring of leaf-trace bundles. This cambium remains permanently active and forms alternately on its inner side collateral vascular bundles and conjunctive tissue; on its outer face it forms a thin layer of phloem or none at all. The plants of the second type develop a ring of primary vascular bundles with normal cambium. The activity of the latter soon ceases, and on the outer face of the primary ring appear in centrifugal order a succession of cambia each of which forms a distinct ring of vascular bundles and intermediary tissue. Classes three and four are types intermediate between the first two.

Morot (4) points out that the two modes of growth described by De Bary may be reduced to one type. The cambium in each case retains its bipolarity, giving rise to xylem on the inside and phloem on the outside.

Fron (5) subsequently states that the stem of *Chenopodium album* increases in diameter by the activity of a normal and pericyclic cambium, and that this cambium produces to the inside xylem and parenchyma and to the outside phloem tissue.

However, notwithstanding the comparatively large amount of work done on the inner structure of the Chenopodiaceae, the origin of the intraxylary phloem, its relation to the cambium and to the xylem of the bundles, remained obscure. It was therefore the primary object of this investigation to study the relation of cambial activity to the development of the anomalous growth. It was also hoped to extend our knowledge of the histology of the vascular tissue, in particular that of the phloem.

Material and Methods

The work was begun during the summer of 1919 at Ft. Lewis, Colorado, and was completed at the Department of Botany, Cornell University. Material taken from the field was studied while fresh. It was found most satisfactory to use unstained hand sections for both anatomical and ontogenetic studies. This method has an obvious advantage over most modern laboratory practice in that it permits the examination of a large amount of material in all stages of development with the least expenditure of time. But for the purpose of checking results and for use in making photomicrographs, representative material was killed in Flemming's weaker solution, embedded, some in paraffin, some in celloidin, sectioned, and stained in the usual manner.

Anatomy

A transverse section of a young stem shows between pith and cortex a circle of separate bundles—the leaf traces. Their number varies, there being even in very young stems as many as twenty. The largest of these traces belong to the lower leaves, the smallest to the primordia of the leaves near the growing apex.

These primary leaf-trace bundles are collateral. The phloem in cross section is a compact oval or oblong mass of tissue (Pl. XVI, *A*), bounded externally and laterally by parenchyma cells which, when still young, contain chloroplasts. The xylem also is definitely set off from the surrounding fundamental tissue. Its first-formed elements are scattered, and only the later formed metaxylem and the secondary elements show a definite arrangement in radial rows. In older sections we notice the development of an interfascicular cambium uniting individual leaf-trace bundles of the primary cycle, and an extrafascicular cambium from which originates a series of collateral bundles and conjunctive tissue. This tissue later lignifies and, together with the xylem of the bundles, forms a compact woody cylinder in which appear embedded small islands of phloem. The origin of this intraxylary phloem and of the conjunctive tissue, together with changes which take place when the tissues mature, will be discussed under the heading "Ontogeny."

No true secondary phloem develops; the true phloem remains restricted to the bundles in a given cycle. A narrow band of pericycle, sometimes only a ring of fibers, separates the vascular cylinder from the cortex. The cortex itself is of only limited extent and rarely more than two to four cells wide. The outer cortex is differentiated into collenchyma and photosynthetic tissue. The former occupies the ridges of the stem while the latter is found in the intervening spaces. The one-celled epidermis is of two types: the epidermal cells external to the collenchyma are elongated and their tangential walls are thickened; those external to the photosynthetic region are thin-walled and nearly isodiametric.

The collenchyma cells are long, pointed, and thickened at the corners only and communicate with one another by simple pits. The cortex and pith are made up of thin-walled, loosely connected parenchyma cells in which are often found druses of calcium oxalate (Pl. XVII, *B*).

Occupying the periphery of the vascular cylinder is a ring of fibers the elements of which are of the usual type but vary in size and diameter of lumen. They are rarely completely united into a closed ring but rather form short bands one to several cells wide. In places the cells of the phloem elements of the vascular tissue abut directly on the fibers; most often, however, a narrow band of pericyclic tissue intervenes.

The elements of the vascular cylinder are in general of advanced dicotyledonary types. The frequent occurrence of transitional forms in xylem and intermediary tissue makes the study of this group of plants especially interesting.

The xylem is made up of porous vessels, fibers, and wood parenchyma, the last named being vasicentric. The vessels are of two general types (Pl. XVI, *C*). The large type, most commonly arranged in uniseriate radial rows (Pl. XVI, *B*), is rectangular with end walls nearly transverse. The small type of vessel shows less definite arrangement; it is more elongated and its end walls are always more or less oblique. The walls of the vessels are heavily pitted. The pits are small, pentagonal, and arranged in alternate rows (Pl. XVI, *C*, *G*). In the small type of vessel, however, the pits may not show the symmetrical form and regular arrangement.

The fibers approach the libriform type. The elements are long and pointed but comparatively thin-walled. The walls are but sparingly pitted. The longitudinal course of the fibers is not absolutely straight in that the ends of the elements diverge obliquely whereby they become partly interlocked, which arrangement gives the wood an especially great toughness.

In the xylem of the leaf-trace bundles we find in addition to the types of elements just described the typical elements of the protoxylem with transition forms to the pitted vessels. The first formed elements of the protoxylem are narrow; the secondary thickenings of their walls are of the nature of loose spirals and wide rings (Pl. XVI, *E*). Much protoxylem, however, is made up of larger elements with secondary thickenings in the form of close spirals. Gradually the arrangement of the elements becomes more definite. The type of element also changes, and instead of close spirals we now find elements with scalariform and sometimes with reticulate walls. Various types of transitional forms are found between the spiral element on the one hand and the pitted vessel on the other. The metaxylem, and of course the wood produced by the cambium, contain only pitted vessels.

The xylem, as we have learned, forms a compact woody cylinder made up of a series of concentric, undulate zones of growth (Pl. XVI, *B*), each zone in turn being a circle of collateral vascular bundles united with one another by intermediary or conjunctive tissue. Separating each zone of growth is a tangential band of parenchymatous tissue of varying width. Narrow, or sometimes broad, bands of parenchyma traverse the xylem in radial direction. These bands usually connect radially the individual zones of growth. Often, however, they pass through several zones, and in those instances closely resemble medullary rays.

In cross section the cells of this conjunctive tissue appear like ordinary parenchyma, but in radial and tangential cuts a great variation in form manifests itself. There are groups of cells made up of the common substitute fiber—a tracheidal element with simple pits. Other elements of this tissue are comparatively short or even isodiametric. Morphologically, then, this tissue is not homologous with rays, though it may function as such.

The phloem is made up of three types of elements: the sieve tube, the companion cell, and the phloem parenchyma. Contrary to the conclusion of earlier investigators (1, 4), the sieve tube is the principal element of this tissue. The tubes form longitudinal series (Pl. XVI, *D*) with occasional anastomosing of the elements of closely connected groups. On the whole, however, the course of the phloem groups is radial-perpendicular, with connections of the elements of the groups taking place only through the leaf gaps formed by the branching of the leaf-trace bundles and their subsequent fusion.

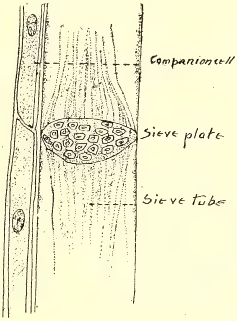

FIG. 1. *Chenopodium album:* Diagrammatic drawing of sieve tube and companion cell.

The sieve tube is of medium size with an average diameter of 14 μ. The end walls are usually slightly oblique, which makes it difficult to observe the sieve plates in strictly transverse sections. There are no sieve plates in the radial walls. The latter, however, are extensively pitted with the companion cells, which, as is usually the case, are connected by simple pits with the neighboring parenchyma cells of the conjunctive tissue. Late in the season the plates of many sieve tubes become covered with callus; this callus formation is most often observed in the phloem of the primary bundles.

ONTOGENY

Almost immediately below the growing point the procambium becomes distinct, forming a concentric band of tissue between cortex and pith. At certain points in this procambium the primordia of vascular strands appear recognizable as such by the small size of the elements and by their granular content. The smallest primordia contain only elements of the phloem. Slightly older groups also contain elements of the protoxylem. The first formed phloem cells are thin-walled, very narrow, and take the typical haematoxylin stain. Sieve tubes become distinct in maturer sections only. The protoxylem elements are also narrow and of the loose spiral type (Pl. XVI, *E*). The groups of phloem and xylem are at first separated by undifferentiated procambium. With enlargement of the group, a cambium develops which later initiates secondary growth.

The procambium surrounding the strands of vascular tissue undergoes active division, causing the primary bundles to become separated and initiating the formation of new bundles in the widening spaces. These new bundles naturally do not extend so far into the pith (Pl. XVII, *D*), and, like the primary bundles, they increase in size through the activity of a cambium.

Gradually the procambium cells between the vascular groups cease dividing. All the elements mature except a single layer which remains meristematic, and which, as an interfascicular cambium, unites the separate bundles into a vascular ring. This cambium layer appears at first only between the larger groups, while later the smaller and more distant groups may also become united to form a part of the primary cylinder.

During the enlargement of the primary bundles an extrafascicular cambium appears (Pl. XVII, *A*, *C*) in the still undifferentiated procambium on the outer face of the primary bundles. This layer of meristematic cells is not formed simultaneously in a given circle, and as a consequence,

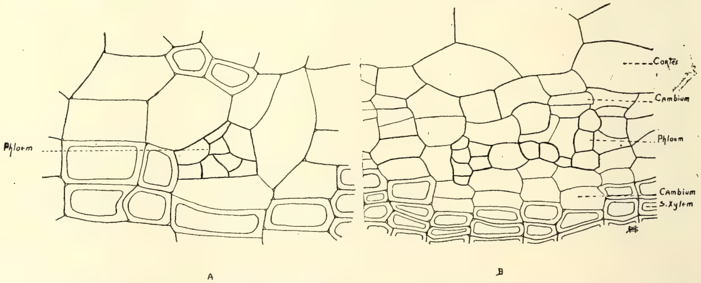

FIG. 2. *Chenopodium album:* Drawings illustrating the mode of origin of the intraxylary phloem. *A*, transverse section of stem showing cambium cells dividing in different planes, forming eight cells which become the phloem of the vascular ring. *B*, transverse section of stem showing the appearance of a new cambium in the parenchyma adjacent to the newly formed phloem groups.

different parts of the ring are found in different stages of development. This condition results in the formation of an undulate circle of cambium. In this cambium, cell division takes place only centripetally, resulting in the formation of a cylinder of tissue consisting of alternate radial segments of xylem and of conjunctive tissue. The cells of this secondary xylem, the large vessels in particular, are arranged in radial rows, differing therein from the primary tissue in which the elements are without any definite arrangement.

After a limited period of activity, certain areas in the cambium, chiefly opposite the large primary bundles, undergo a change of function. One or two cambium cells divide rapidly in different planes forming from two or three to ten cells (fig. 2, *A*, *B*; Pl. XVII, *E*, *F*).

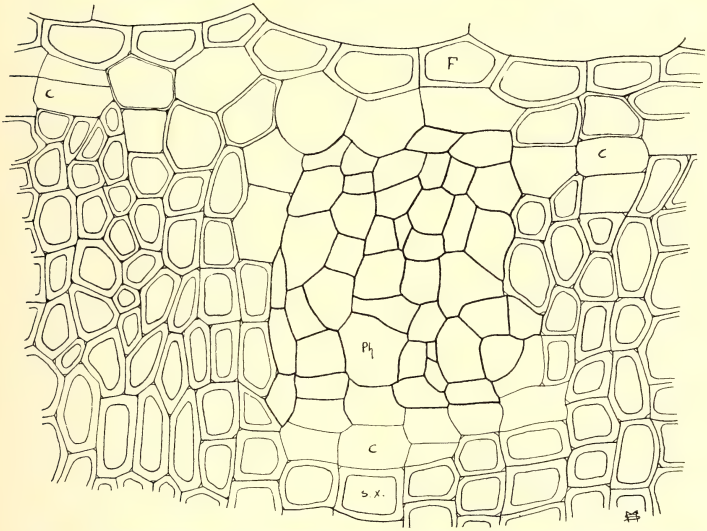

FIG. 3. *Chenopodium album:* Transverse section of stem showing mature phloem group. The appearance of the new cambium segment above the group is belated. The cambium at either side of the phloem group has divided very actively and has thus caused the phloem to be completely embedded in xylem. (*C*, cambium; *F*, fibers; *Ph*, phloem; *s.x.*, secondary xylem.)

These cells become the phloem of the just-formed vascular ring. We thus have a ring of xylem with segments of parenchymatous conjunctive tissue with a number of separated phloem strands on the outside. Occasionally the portions of cambium behaving in this manner are not used up in this process but may form a small amount of xylem toward the inside after the completion of phloem formation. These segments of cambium disappear, as such, since they mature into vascular tissue. The ring of cambium is thus broken up.

The cells of the cambium which are not concerned with the formation of these phloem groups are dividing very actively meanwhile, so that the cambium ring becomes undulate, the phloem groups occupying the depressions. Very soon after the initials of these phloem groups appear, a cambium forms in the parenchyma adjacent to the outer face of these groups (fig. 2, *B*). This new cambium becomes connected laterally with the cambium ring of the vascular cylinder. Sometimes the appearance of these new cambium segments is belated, and not until the unequal activity of the cambium ring has produced the undulate appearance and the depressions does a cambium layer appear at the outer face of each new group (fig. 3). In such a case the cambium formation begins at the margin of the depression, advances laterally, and when united undergoes reciprocal tangential division, thus giving rise to xylem and parenchyma which mature in the normal manner.

The formation of new cambium initials which mature into groups of phloem completes the growth of a zone of thickening which is succeeded by a new similar zone. The same process is repeated, and thus arise the undulate zones of vascular tissue so characteristic of the members of the Chenopodiaceae.

Conclusions

The study of the anatomical features of the vascular tissue of the stem in part confirms and in part modifies and extends the results obtained by earlier investigations. In the discussion of the histological features of the phloem it was shown that sieve tubes and not phloem parenchyma make up the larger portion of that tissue. Why earlier investigations limit or even deny their occurrence is hard to understand. Even Gheorghieff in his detailed anatomical researches of the group simply states: "Die Phloempartie ist vorwiegend aus parenchymatischen Elementen zusammengesetzt. Siebröhren habe ich nur selten gefunden." It must be admitted, however, that the elements of the phloem are comparatively small, and that the sieve tubes especially are narrow and easily mistaken for plasma-rich cambiform elements unless staining reactions show the sieve plates or the callus deposits over the plates. The typical staining reaction of this substance is a further aid in identifying the sieve tubes.

The elements making up the conjunctive tissue exhibit such a variety in form and arrangement that they could not be conceived of as ray cells in the morphological sense. That they may function, however, as rays is not at all unlikely.

Above all, however, this study has shown that the anomalous growth of the stem is produced by a periodically acting cambium which is progressively renewed at places where new phloem groups originate. In the development of each individual zone of growth, the xylem of the bundle is formed first, its formation being followed by a change in the activity of the initial strand on the outer face of the cambium; from the active division of this strand the phloem is produced. There is little if any new xylem added to the vascular ring in those places where phloem initials originate, for the xylem of the bundles, as has already been shown, is developed before any of the phloem matures. The cambium then does not exhibit the unipolarity which De Bary claimed for the group to which *Chenopodium album* belongs; it is always bipolar in restricted regions in that it gives rise to normal tissue elements on either side.

SUMMARY

1. The anomalous stem structure of *Chenopodium album* is produced by a periodically active cambium which forms xylem centripetally throughout its extent and phloem centrifugally in restricted regions. Where phloem is formed the cambium is "used up." The continuity of the cambium ring is maintained by the formation of new portions outside the phloem groups.

2. The phloem of a secondary zone of growth is produced after all or most of the xylem has been formed. It is the normal product of the cambium and only belated in its development.

3. The intermediary or conjunctive tissue is not ray tissue in the morphological sense though it may function as such.

4. The chief element of the phloem is the sieve tube with its companion cell. Phloem parenchyma is of only secondary importance.

5. The stem structure shows in its ontogeny a striking similarity to the structure of the root of the sugar beet, a developmental study of which is contained in De Bary's "Comparative Anatomy."

LABORATORY OF PLANT PHYSIOLOGY,
CORNELL UNIVERSITY.

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2. **Sanio, C.** Vergleichende Untersuchungen über die Anordnung der Elementarorgane des Holzkörpers. Bot. Zeit. **21**: 389. 1863.
3. **De Bary, A.** Comparative anatomy of the phanerogams and ferns, p. 594. 1877.
4. **Morot, L.** Recherches sur les péricycles. Ann. Sci. Nat. VI. Bot. **20**: 276. 1885.
5. **Fron, G.** Racine et tige des Chénopodiacées. Ann. Sci. Nat. VIII. Bot. **9**: 157. 1899.

EXPLANATION OF PLATES

PLATE XVI

A. Cross section of a large primary bundle showing arrangement of primary and secondary xylem; position and extent of phloem groups.

B. Cross section of part of mature stem showing undulate appearance of the zones of growth; extent and position of the conjunctive tissue and the radial arrangement of the large vessels.

C. Longitudinal section of xylem showing both types of vessel.

D. Radial section of stem showing size and longitudinal course of the phloem.

E. Radial section of primary xylem showing spiral and ringed elements.

F. Radial section of phloem showing the sieve tubes with companion cells and phloem parenchyma.

G. Radial section of xylem showing (from left to right) fibers, vessels, and conjunctive tissue.

PLATE XVII

A. Cross section of part of young stem showing the appearance of an extrafascicular cambium above a phloem group.

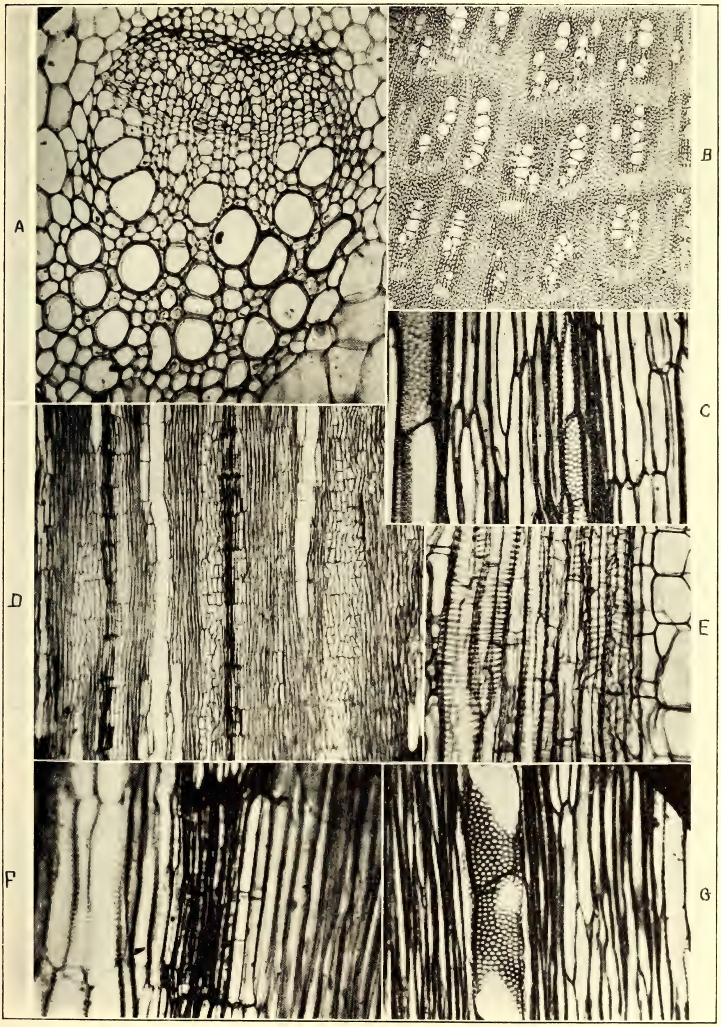
B. Cross section of a more mature stem showing the same condition as in *A.* Druse of calcium oxalate in cell of cortex.

C. Section of primary bundles of stem. Above the bundles an extrafascicular cambium has developed which is several rows wide.

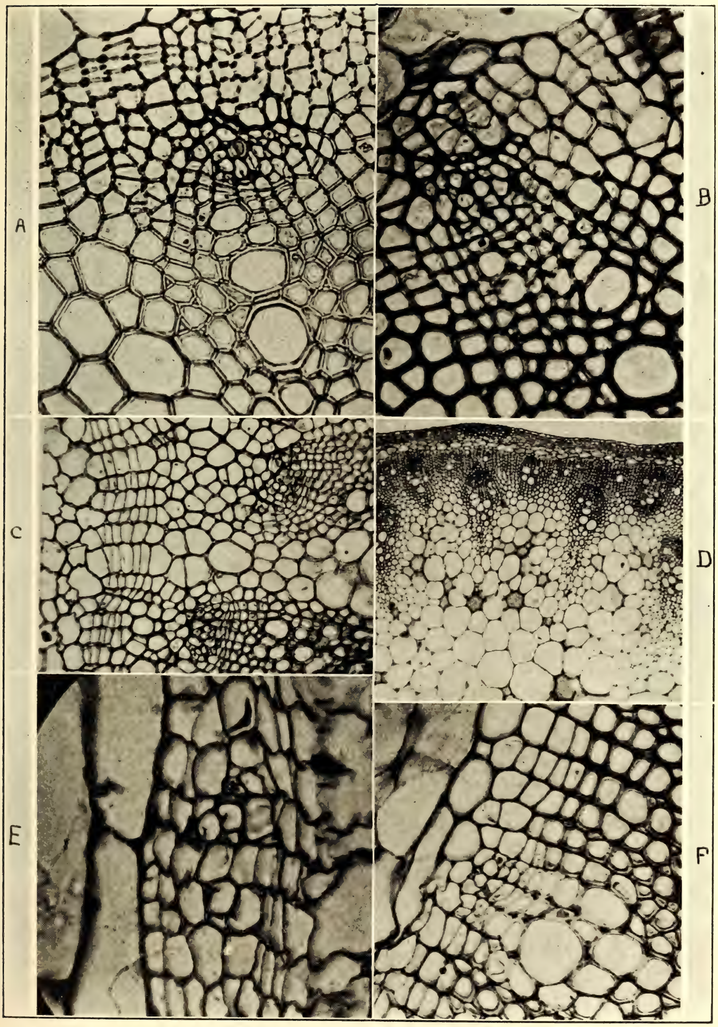
D. Cross section of young stem showing several primary bundles and the first zone of thickening.

E. Section of vascular strand initial. Extrafascicular cambium has developed above the newly formed phloem group.

F. Section through annual zone of growth showing the development of an initial strand of phloem.



ARTSCHWAGER: ANATOMY OF CHENOPODIUM ALBUM.



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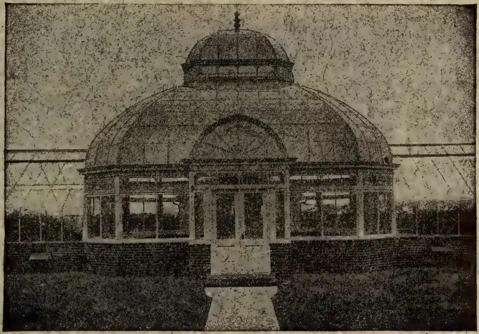

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MEASUREMENT OF THE CATALYTIC POWER OF CATALASE

L. G. M. BAAS BECKING AND H. C. HAMPTON

I. INTRODUCTION

In this paper we propose to describe a new method and a new principle of determining the strength of catalase action. We do not endeavor to sum up all the literature existing, the excellent works of C. Oppenheimer[1] and H. Euler[2] having made this unnecessary. Of the literature which has appeared since 1909 we have taken only such work into account as showed an immediate connection with ours. Therefore we merely mention the elaborate work of G. B. Reed.[3]

The present problem originated from a proposed research on autofermentation in *Cannabis sativa* L. The first enzym to be dealt with was catalase. There were many difficulties to be overcome before we could start the work. The easiest way is to take commercial peroxide and let it act on crude plant juice, determining either the oxygen discharged by the method described by H. H. Bunzel[4] or the peroxide decomposed by means of titration with permanganate of potassium—the latter the method of nearly all other authors. With both methods we made determinations, but were very soon convinced that we were not ascertaining the actual strength of the enzym. One aim of the present paper, therefore, is to attempt to prove the inadequacy of the existing methods.

The value of a new method depends entirely on three factors: (1) The purity of the enzym; (2) the purity of the peroxide; and (3) the way of determining the action of the enzym on the peroxide. We took care only of the third factor in our experiments, though for the sake of completeness we mention all three. The first factor may vary according to the different aims of the research.

For studies in kinetics (R. O. Hertzog[5]), the enzym must be free from crystalloid, from peroxidase, and from impurities. For physiological research the methods of precipitation, be it with strong alcohol, inorganic salts or lead acetate, or by dialysis and centrifuging, enfeeble the enzym to

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[1] Die Fermente, Zehnte Aufl Leipzig, 1909.

[2] Grundlagen und Ergebnisse der Pflanzenchemie, 2ter Teil. 1909.

[3] Bot. Gaz. 1915–1918.

[4] Journ. Biol. Chem. **20.** 1914.

[5] Zeitschr. Physik. Chem. **41.** 1904; Oppenheimer, Part II.

[The **Journal** for June (**7**:223-260) was issued July 29, 1920.]

a marked degree and are therefore to be avoided as much as possible. All authors, however, agree that, although the influence of accompanying salts may not be very great, damage by acid is considerable (G. Senter[6]). Therefore the physiologist must take care to neutralize the juice. A neutralization with Na_2CO_3 worked in our experiments very satisfactorily. In experiments in which the same juice is used for several days, a few drops of toluol must be added as a preservative.

The impurity of commercial peroxide should not be overlooked. It contains acid (even the perhydrol of Merck) which must first be neutralized. Also, most of these peroxides contain a certain amount of acetanilide (±1/15 percent). G. Senter has proved that aniline is poisonous to catalase. Therefore it is better to work with purified peroxide (methods by G. Bredig[7]). However, we shall try to show that, working with our method, the results are not greatly influenced by the impurities of the enzym or of the peroxide. We worked in these preliminary experiments with commercial 10-volume peroxide and freshly prepared, mostly undiluted, plant juice, neutralized during the grinding. We shall try the same method later on with purified chemicals and enzym.

The third factor, that of the method of determining the quantity of oxygen liberated or of peroxide decomposed, is the most important one. There are three methods for the quantitative measurement of catalase.

1. The method of Palladin (cited by A. Kasanski[8]) consists in measuring the height of the foam developed during the reaction. There is, however, no sufficient ratio between the intensity of the phenomenon and catalase activity. The purer the enzym, the smaller the volume of foam, etc.

2. Titration with potassium permanganate. This is the method used by nearly all authors, but we have considerable doubt that it will serve its purpose. In the first place, organic compounds of different kinds oxidize permanganate. Therefore the press juice of itself, has a certain oxidizing power. This power is difficult to measure, not only because the end point of the reaction (a permanent red color) is difficult to observe, but also because the fluid is often so much colored that color reactions cannot be measured. These facts are stated by P. Waentig and A. Steche[9] and by W. Issajew.[10] The latter does not mention his method, so that the value of his results could not be judged. Permanganate shows this uncertain end point also with all kinds of organic salts (citrates, malates, tartrates) as we were able to prove. Therefore it is not surprising that even excellent scientists like Bach sometimes made considerable errors with the titration method (A. M. Clover[11]). Notwithstanding our working with the usual

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[6] Zeitschr. Physik. Chem. **51**. 1905.

[7] Zeitschr. Physik. Chem. **31**. 1899.

[8] Biochem. Zeitschr. **39**. 1912.

[9] Zeitschr. Physik. Chem. **72**, **76**, **79**, **83**. 1911–1915.

[10] Zeitschr. Physik. Chem. **42**. 1905.

[11] Amer. Chem. Journ. **29**. 1904.

precaution, we distrust the value of the numbers we obtained with the permanganate method.

3. We come now to the manometrical methods. These lack the advantage of the titration method in working under normal pressure. Furthermore, the fluid may reach the unfavorable condition of over-saturation with gas (P. Waentig and A. Steche, *l.c.*). Of the authors whose work has been done by this method we name: C. H. Appleman,[12] W. W. Bonns,[13] H. H. Bunzel (*l.c.*), W. E. Burge,[14] C. Foa,[15] W. B. Magath,[16] W. Zaleski and Anna Rosenberg.[17] The chief objections to this method are: (1) The pressure becomes higher during the reaction. We feel justified in disregarding the effect of over-pressure as our check experiments have shown this to be negligible. (2) The solution contains a great part of the oxygen. This is true only for narrow vessels, in which the surface is small in proportion to the volume of air. Even in the apparatus of H. H. Bunzel (*l.c.*) we feel that there is danger of the fluid becoming oversaturated. Bunzel tried to avoid this danger by shaking. But R. O. Hertzog (*l.c.*) cites a list of cases in which enzyms are destroyed by shaking. For instance, P. Waentig and A. Steche (*l.c.*) proved the destructive action of shaking on catalase.

It seemed clear to us, therefore, that if we chose the lesser of two evils, namely the manometrical method, we should take a container with a very broad bottom and a shallow layer of fluid. Experiments have shown us that the effect of shaking on the exchange of gas in such a column is minimal. In the short time of the catalase reaction the enzym is not injured by shaking. In more prolonged experiments (with oxidases for instance) it may be. There is another advantage in experimenting with a shallow layer of fluid since R. O. Hertzog (*l.c.*) proved that the catalase reaction is subject to the laws of diffusion, which is the most complete in thin layers.

II.

One more important criticism of nearly all methods of enzym determination is possible. To detect the fault we must start at the very beginning, at the definition of the word *enzym*. An enzym is a substance that *changes the velocity* of a reaction. Peroxide of hydrogen will decompose spontaneously but slowly. It will oxidize a certain amount in one month. Catalase changes the reaction time from one month to one minute. The only method theoretically justified would therefore be to determine the the time in which a reaction is completed under the influence of an enzym. That time is the measure of the enzym action.[18] If the reaction is monomolecular, and follows the law of mass action, the well-known formula of van't Hoff will apply:

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[12] Bot. Gaz. **50**. 1910.

[13] Ann. Mo. Bot. Garden **5**. 1918.

[14] Amer. Journ. Physiol. **44**. 1917.

[15] Biochem. Zeitschr. **11**. 1908.

[16] Journ. Biol. Chem. **24**. 1918.

[17] Biochem. Zeitschr. **33**. 1911.

[18] On the assumption that the reaction time *without* enzym is *very much* greater than that time *with* enzym.

$$\frac{dx}{dt} = k(a - x)$$

$$\text{in which} \begin{cases} a = \text{available amount,} \\ x = \text{decomposed amount,} \\ t = \text{time,} \\ k = \text{reaction velocity.} \end{cases}$$

Integration of this form $k = \frac{1}{0.4343t} \log \frac{a}{a - x}$ enables us to find the reaction velocity from a single determination. Concerning catalase a great variety of opinions exists in regard to the constancy of the reaction velocity. If the reaction velocity were proved to be practically constant, we should find, if *x* approaches its maximum value (let us say $\frac{999}{1000} a$):

$$k = \frac{3}{0.4343t}$$

or, k will be inversely proportional to t. In this case only would one be justified in measuring the so-called reaction-velocity, taking this as a comparative number for the "real" reaction-velocity, *i.e.*, $\frac{1}{\text{reaction time}}$. Excepting Bredig, who first called attention to this fact, F. A. F. C. Went[19] is the only author, so far as we know, who has tried to determine directly the time in which a reaction took place. He studied starch hydrolysis by the enzym of *Aspergillus niger*. His numbers are interpolated but still show marked properties.

In all cases, more or less scattered determinations (see, for example, figures in W. M. Bayliss[20] on glycerol-glucoside) must furnish the basis for the calculations. In our special case of catalase action, an *autographic method* which marks the time in which the reaction is ended offers a solution of this difficulty. Furthermore, this gives us opportunity to collect a far greater number of figures. As we learned after we had worked out the apparatus, the idea of an autographic record was not new.

C. Foa (*l.c.*) used a Mosso-plethysmograph and a revolving drum with soot paper to determine the action of different phenols on oxidase. He published his graphs without using them for calculation. A. Schultze (cited by Foa), used a self-recording manometer for measuring the CO_2 output in yeast activity. M. Antropoff studied autographically the periodical decomposition of peroxide by mercury. (Stephane Leduc[21] has explained his results in a peculiar way.)

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[19] Verh. Kön. Akad. Wet. Amsterdam **27**. 1918.

[20] General physiology, 2nd edition. London, 1917.

[21] Théorie physico-chimique de la vie. Paris, 1910.

III.

We shall start now with the description of our own experiments.

We used a rather large reaction vessel connected with a manometer by a ground joint. The vessel was closed by a ground stopper. Joint and stopper were fastened on the bottle with strong rubber bands. On the ground stopper was sealed a small vessel with two holes. The small vessel contained the peroxide, while the larger held the enzym. The idea was borrowed from Haldane's well known apparatus for blood-gas determination. By turning the vessel in a plane perpendicular to the paper, the peroxide flowed from the smaller vessel into the larger one. In this plane the vessel could also be shaken. If the fluid contained catalase, oxygen would be liberated. This would increase the pressure in the vessel and the mercury column in the manometer would rise.

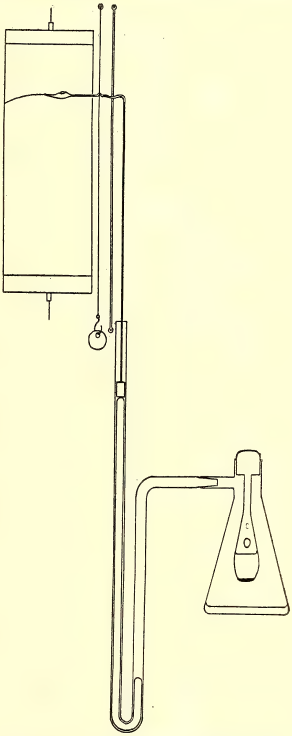


FIG. 1. Self-recording manometer with modified Haldane apparatus.

The autographic writer was simple to make. We had at least four methods from which to make our choice: (1) Transfer of the movement by levers; (2) transfer of the movement by air (Marey, Buisson); (3) direct record of mercury level by sensitive paper; (4) direct transfer.

The second method would not be the most efficient in our case, for, like the first method, it would record the results either enlarged or reduced. In the case of the third method we could not use coordinate paper. Therefore, we transcribed the pressure by means of a wooden float which carried a thin glass rod on which a glass pen was sealed at right angles to the rod. This we kept in position by a glass slide-bar and a weighted hair. This simple arrangement made it possible to register differences of $\frac{1}{20}$ mm. in the mercury level. The friction had an effect of $\frac{1}{10}$ mm. We therefore always calculated the records in millimeters. The efficient speed for the rotating drum was in our case one revolution in eight minutes for a diameter of 10 cm. The pen went over the distance of 1 mm. in $\frac{13}{8}$ seconds. The speed should be slower in the case of purer enzyms in which the action is feeble. The flask stood inside the thermostat and could be shaken from the outside by means of a handle.

We obtained in normal cases a curve of the shape shown in figure 2.

At A (fig. 2) the peroxide is in contact with the enzym; at B the reaction begins; at C the reaction is completed. The distance A–B existed always and was not caused alone by the transportation time of the pressure. The distance A–B became greater when we used smaller quantities of enzym. This fact, namely, that oxygen is not immediately discharged, is mentioned only by P. Waentig and A. Steche (*l.c.*) With the use of the non-autographical methods, and especially with that of the titration method, this fact nearly always escapes observation. We saw the latency time (A–B) manifested, when the titration method was used, only in dilute enzym solutions. The small bubbles of oxygen were formed sometimes one minute after the beginning of the reaction. *It is clear, therefore, that the titration method not only is unfit to give us the end point of the reaction; it is unfit also to give us the moment of the beginning.*

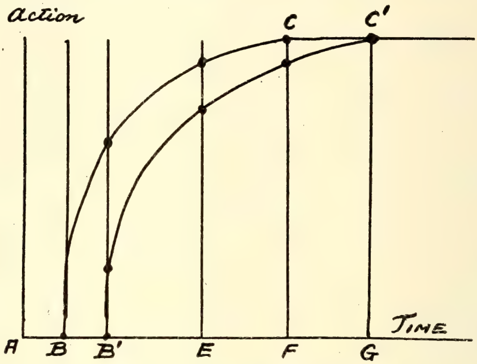
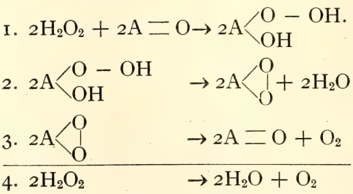


FIG. 2. For explanation see text.

The differing length of A–B with different enzym dilutions gives us a hint that the decomposition is caused by two successive reactions (A. Bach[22]), as follows:



1. $2H_2O_2 + 2A = O \rightarrow 2A\langle\begin{matrix}O-OH.\\OH\end{matrix}$ This unstable compound decomposes:

2. $2A\langle\begin{matrix}O-OH\\OH\end{matrix} \rightarrow 2A\langle\begin{matrix}O\\|\\O\end{matrix} + 2H_2O$ This peroxide is decomposed:

3. $2A\langle\begin{matrix}O\\|\\O\end{matrix} \rightarrow 2A = O + O_2$ Summing up, we obtain

4. $2H_2O_2 \rightarrow 2H_2O + O_2$

---

[22] Chem. Ber. 36–42. 1904–1908.

This reaction (4) would be true when $A - B = 0$ seconds.

This is the reaction given by most authors. G. Bredig (*l.c.*), however, doubted the value of this equation. He found the reaction velocity constant. Therefore, he argues, the reaction must be monomolecular, and follow the scheme:

$$H_2O_2 \rightarrow H_2O + O$$

But the oxygen liberated is not atomic oxygen; it is not ionized, but molecular. T. H. Kastle and A. S. Loevenhart[23] defend on this ground the validity of the bimolecular equation. We are not able to follow their criticism of Bredig's work, and we will only remark that in the case in which $A - B > 0$ the whole controversy seems to be solved. In fact, the first two reactions in the scheme of Bach are monomolecular.

IV.

We will compare now the action of two different quantities of enzym. The line A–B′–C′ (fig. 2) gives the action of the smaller quantity. What method must we follow to find out the ratio of their strengths?

1. *Reaction velocity.* The more accurate investigations deal with reaction velocity. We will show that the enzym is destroyed during the reaction (see below). Therefore the reaction velocity diminishes (sometimes very slightly) as nearly all authors have shown. (Issajew, *l.c.*, however, finds a constancy to the third decimal.) *The reaction velocity is therefore a misleading test for the strength of an enzym.*

2. *Amount of peroxide decomposed.* This method, though much used, has very little value, as figure 2 will demonstrate. A determination of the ratio in strength between A–B–C and A–B′–C′ would give:

At B′, 10:5;
E, 10:7;
F, 10:9;
G, 10:10.

Still we find in the literature on the subject, expressions like this: "There is three times as much catalase in the body wall of *Ascaris suum* as in the leg muscles of *Rana pipiens*" (Magath, *l.c.*).

3. A better method is the direct measurement of the reaction time (see above, II). This is possible only with a self-recording apparatus.

We prepared our materials by the method thus described. The tops of female hemp plants were ground in a meat-grinder with a small amount of powdered Na_2CO_3. The ground substance was then squeezed in a fruit press. The turbid fluid obtained is very stable and still strongly active after the lapse of fourteen days. The determinations all took place at 20° C. The peroxide was the usual commercial 10-volume H_2O_2, which contains acetanilide enough to damage the enzym during the reaction (see below). We expect to repeat the experiments under standard conditions and ask the reader therefore to consider this paper as a preliminary account.

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[23] Amer. Chem. Journ. 29. 1903.

The time in which the reaction on 2 cc. peroxide was completed was in one case:

For 4 cc. extract, 15 mm. (15 × $\frac{13}{8}$ sec.);
for 3 cc. extract, 21 mm. (21 × $\frac{13}{8}$ sec.);
for 2 cc. extract, 29.5 mm. (29.5 × $\frac{13}{8}$ sec.);
for 1 cc. extract, 59 mm. (59 × $\frac{13}{8}$ sec.);
for $\frac{1}{2}$ cc. extract, 118 mm. (118 × $\frac{13}{8}$ sec.).

We can state that the reaction time is inversely proportional to the amount of enzym, E (amount of enzym) × T (reaction time in units of $\frac{13}{8}$ seconds) thus being constant.

Again:

For 4 cc. extract, $E \times T = 60$;
for 3 cc. extract, $E \times T = 63$;
for 2 cc. extract, $E \times T = 59$;
for 1 cc. extract, $E \times T = 59$;
for $\frac{1}{2}$ cc. extract, $E \times T = 59$.

In another case we obtained these results:

For 4 cc. extract, $E \times T = 24$;
for 3 cc. extract, $E \times T = 27$;
for 2 cc. extract, $E \times T = 26$;
for 1 cc. extract, $E \times T = 25$;
for $\frac{1}{2}$ cc. extract, $E \times T = 24.5$.

These numbers strikingly show the value of the autographical method.

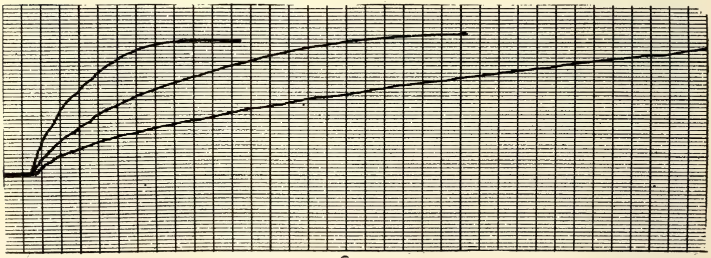

FIG. 3. Autogram. Effects of different quantities of enzym.

Figure 3 gives still another proof for 3 curves, taken with 4, 2, and 1 cc. of extract respectively, and 4 cc. 10-vol. H_2O_2. Different amounts of peroxide change the time in the same manner. Figure 4 will illustrate this.

4 cc. of extract react with:

	Time in "mm."	T / peroxide	
4 cc. 10-vol. peroxide	45	11.3	$\frac{(45)}{4}$
3 cc. 10-vol. peroxide	34	11.3	$\frac{(34)}{3}$
2 cc. 10-vol. peroxide	22	11.	$\frac{(22)}{2}$
1 cc. 10-vol. peroxide	10	10	$\frac{(10)}{1}$

The times are proportional to the amount of peroxide.

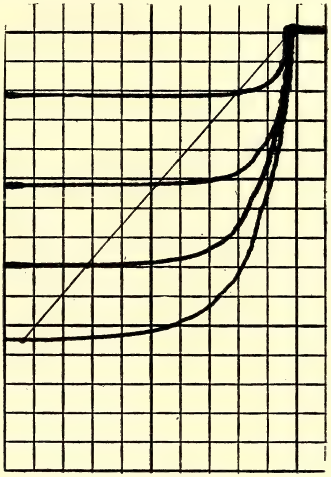

FIG. 4. Autogram. Effects of different quantities of peroxide.

To compare these results with the reaction velocities, we used the following method: We imagined the assymptote extended to the ordinate axis. Then the quantity (a − x) of the formula

$$\frac{dx}{dt} = c\,(a - x)$$

will be the distance from a given point of the curve to the assymptote. We call it D. We can write for a point of the curve P:

$$tg\alpha_P = K_P D_P, \text{ or } K_P = \frac{tg\alpha_P}{D_P},$$

α_P being the angle between the tangent at the point P and the time axis.

We had only to measure α_P and D_P and we could read immediately on the slide rule the resulting K_P. We averaged a great number of K's taken from different curves. We also calculated the probable error. It proved to affect the units only. This must be taken into account.

Extract in cc.	Peroxide in cc.	Number of Experiments	Reaction Velocity	$\frac{\text{Peroxide} \times K}{\text{Enzym}}$
½	2	4	41×10^{-4}	164×10^{-4}
1	2	7	110×10^{-4}	220×10^{-4}
2	2	11	257×10^{-4}	257×10^{-4}
2	4	4	109×10^{-4}	218×10^{-4}
3	2	4	396×10^{-4}	264×10^{-4}
4	2	1	535×10^{-4}	263×10^{-4}
4	4	4	261×10^{-4}	261×10^{-4}
1	4	4	47×10^{-4}	188×10^{-4}

Considering the irregularity of the curves due to the poor clockwork and the possible differences in strength of the enzym solutions, $\frac{PK}{E}$ is a fairly approximately constant number. But it would take 39 determinations of this sort to prove what one determination of reaction time gave us, namely, that the reaction follows the law of mass action.

We tried to compare the curves obtained with mathematically constructed logarithmic lines. (Kapteyn used a similar method for Gaussian curves). We constructed several lines

$$t = c \log \frac{a}{a - x}$$

for c varying from 0.1 to 2 and a = 2 cm. We found in one case:

Calculated from Curve	Amount extract	C × E
0.15	4 cc.	0.6
0.2	3 cc.	0.6
0.3	2 cc.	0.6
0.6	1 cc.	0.6
1.2	0.5 cc.	0.6

Perhaps this method will be found to be the most practical and accurate.

The line A—B in which, according to our idea, the first part of the reaction must take place, becomes long enough to be measured in very feeble enzym concentrations only. Figure 5 shows curves run with 3 cc. peroxide and 4, 3, 2, 1, 0.5, 0.2, 0.1, and 0.05 cc. extract respectively, all diluted to 4 cc. fluid. The reaction started at the thick vertical line. The latency time caused by the apparatus (fig. 5) seems to be $2 \times \frac{13}{8}$ seconds. So we had to subtract 2 from the length A–B.

Latency time × *amount of enzym* seems to be more or less constant. But to draw conclusions from these facts seems premature.

Cc. extract	A–B	A–B Calculated for E × (A–B) = 1.5	E × (A–B)
4	Not measurable	0.38	—
3		0.5	—
2		0.75	—
1		1.5	—
0.5	1*	3	—
0.2	7	7.5	1.4
0.1	15	15	1.5
0.05	32	30	1.6

* Out of place.

V.

There is still one assumption which we have not proved. How do we know that the reaction is finished at the point C of our curve (fig. 2), where the curve becomes assymptote to the time axis? To determine this we have only to calibrate our flask. If the volume of the container to the level of the mercury is V_1, the volume of the peroxide V_H, and that of the extract V_E, the remaining volume before the beginning of the reaction will be $V_1 - (V_H - V_E)$. After the liberation of oxygen the mercury is forced down a cm., the volume of 1 cm. to be V_c cc., so the volume after the reaction will be

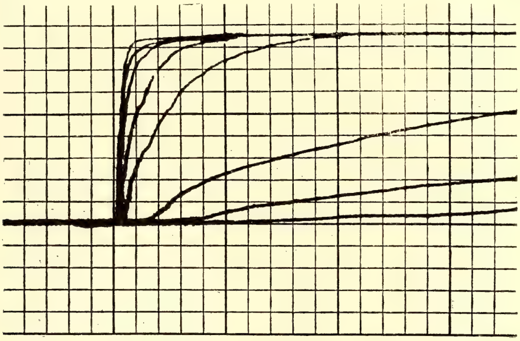

FIG. 5. Autogram showing latency times.

$$V_1 = (V_H + V_E) + aV_c.$$

If the temperature is constant during the experiment, we can use the simple formula of Boyle. If the pressure before the experiment be H cm. mercury, it will be (H + 2a) after the reaction. We get for the volume after the reaction:

$$V_2 = \left\{ \frac{V_1 - (V_H + V_E) + aV_c}{H} \right\} (H + 2a)$$

or the oxygen produced

$$V_2 - \{V_1 - (V_H + V_E)\} = \frac{a}{H}[2\{V_1 - (V_H + V_E)\} + V_c(H + 2a)].$$

In one case we found:

$$\left.\begin{array}{lcl} V_1 &=& 272.19 \\ V_H &=& 2 \\ V_E &=& 2 \\ a &=& 2.3 \\ H &=& 76 \\ V_c &=& 0.29 \end{array}\right\} \begin{array}{l} \text{Oxygen discharged:} \\ \frac{2.3}{76}[2 \times 268.19 + 0.29 \times 80.6] = 17.2 \text{ cc.} \end{array}$$

Now the original peroxide was supposed to be 10-volume. We checked the experiment by titration at the same temperature and found 8.7-volume. This signifies that 2 cc. peroxide would yield 17.4 cc. oxygen.

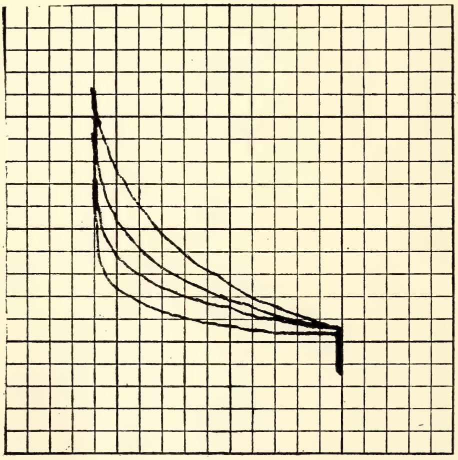

FIG. 6. Autogram showing the influence of successive doses of peroxide.

Thus we see that the autographic method can be used to determine the strength of a peroxide solution.

We can make this clear with an experiment done with 7.5-volume peroxide and 4 cc. extract. The autographic record showed:

Amount Peroxide in cc.	Final Pressure in cm.	cc. Oxygen Calculated From Titration	cc. Oxygen Calculated From Formula
1	1.2	7.5	8.2
2	2.2	15.	16.2
3	3.3	22.5	23.9
4	4.2	30.	28.2
5	5.6	37.5	37.3
6	6.8	45.	45.
7	8.	52.5	52.5
8	9.1	60.	59.6

Successive doses of 4 cc. peroxide on the same 4 cc. extract had the results shown in figure 6. There are two explanations possible. Either (1) the dilution of the solution affects the strength of the enzym, or (2) the enzym is "poisoned" by the peroxide.

We now made a determination of the influence of dilution by means of the titration method. From the results of this experiment we may conclude that the action of catalase does not vary with its dilution or with the quantity of the peroxide, but only with the absolute quantity of the enzym itself.

The results were:

Constitution of the Enzym Solution	Peroxide	Percent Peroxide Decomposed after 1 Minute
1 cc. extract, 0 H_2O	2 cc.	34.6 %
1 cc. extract, 3 H_2O	2 cc.	29.8 %
1 cc. extract, 8 H_2O	2 cc.	33.3 %
1 cc. extract, 15 H_2O	2 cc.	30.9 %
1 cc. extract, 24 H_2O	3 cc.	30.3 %
1 cc. extract, 35 H_2O	2 cc.	31.8 %
1 cc. extract, 48 H_2O	2 cc.	32.9 %
1 cc. extract, 63 H_2O	4 cc.	26.9 %

So only the second assumption is valid, the enzym is destroyed by the peroxide.

The reaction times of the successive amounts were:

	Time	Strength = $\frac{1}{\text{time}}$
1st dose	52 (× 1 5/8 sec.)	100
2d dose	58	89
3d dose	65	80
4th dose	75	69

± 10 percent of the enzym is destroyed during every successive reaction. This decrease in the reaction velocity supports the unproved assumptions of Bredig (*l.c.*).

The influence of alkali is very marked. Enzym solutions neutralized with Na_2CO_3 hold their power for days. Even neutralized hemp powder that had been dried for two weeks showed marked activity. There is a strong possibility that the alkali works as a "peptisator" on the enzym. Many peptisators are known in colloid chemistry, alkali acting very strongly on albuminoids (Graham). The protein character of catalase is probable (Waentig and Steche, *l.c.*). The assumption of an α and a β catalase, proposed originally by O. Loew[24] would in that case be superfluous (compare E. Pozzi-Escot[25]) and Appleman (*l.c.*). The activity of the catalase declines very slowly on filtering, especially if the solution has been previously neutralized. In the latter case the activity decreased only 8 percent.

Unneutralized juices lose their catalytic power very soon. We found, for instance, in one case:

	Standing	Reaction Time	Strength
2 cc. extract neutralized..............	5′	46 units	100
2 cc. extract unneutralized............	5′	85 units	54
2 cc. extract unneutralized............	10′	151 units	31
2 cc. extract unneutralized............	120′		0

There is evidence that this reaction follows also a logarithmic line.

Attempts to prepare the enzym in pure condition have failed. Unlike peroxidase, catalase adheres with a great tenacity to the alcohol precipitate.

We have refrained in the foregoing from discussing the physiological questions suggested by or even suggesting our work, for such a research can start only after the methods are worked out satisfactorily.

SUMMARY

1. A review is given of the literature concerning the question. Difficulties and inaccuracies in several methods are pointed out.

2. According to the definition of an enzym, the reaction time is the only valid index of its strength. This strength can best be measured by an autographical method.

3. An autographical method is given. The method shows the evidence of two successive reactions.

4. The enzym is more or less injured or destroyed during the reaction. In most reactions the time is too short to influence markedly the logarithmic curve.

5. The method given is adapted to determine the strength of a peroxide solution.

6. There is evidence that the two different catalases are different degrees of peptisation of the same substance.

We wish to express our grateful appreciation of the encouragement which Professor G. J. Peirce, by his criticism and suggestions, has given us. We also are indebted to Professors L. L. Burlingame and S. W. Young for valuable advice.

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EARLY STAGES IN THE DEVELOPMENT OF CERTAIN PACHYPSYLLA GALLS ON CELTIS

B. W. Wells

One of the most interesting angles from which the insect gall problem may be attacked is that involving the early stages of gall formation. The present study is a morphological one dealing with the beginning stages of two Pachypsylla (Fam. Psyllidae of the Hemiptera) galls as they develop on the leaves of the hackberry (Celtis).

The Galls

The galls concerned are common ones in their respective regions.

Pachypsylla mamma Riley is found on *Celtis occidentalis* L. It is a hemispheric to subcylindric outgrowth projecting from the under side of the hackberry leaf (Pl. XVIII, fig. 2). Above, on the opposite side, is a prominent depression, in the center of which is a minute conical process. Interiorly there is an inverted dome-shaped larval cavity lined by soft nutritive tissue; this latter in turn is invested by masses of hard, "protective" sclerenchyma. For a detailed discussion of the adult gall, the reader is referred to an earlier paper of the author's (8).

Pachypsylla asteriscus Riley occurs on *Celtis mississippiensis* Bosc. (fig. 1, *c*, and fig. 7). It projects from both sides of the leaf, the part on the under side assuming the shape of a much abbreviated Convolvulus corolla, the part above consisting of a slender, straight process. These processes are attached to a blister-like enlargement in the plane of the leaf, in the interior of which is the lens-shaped larval chamber. Well defined layers of sclerenchyma occur in this gall, bounding the nutritive tissue.

Both of these galls are prosoplasmas or "higher" galls characterized by definite constitution and growth period and possessing highly specific forms and differentiation structures, which latter unusual characters are induced to appear under the action of the specific stimulus developed by the insect larva.

Earlier Work

No ontogenetical studies of the beginning stages of the galls formed by this genus of insects have heretofore been made. A few studies, however, have been made on the galls of other genera of the Hemiptera which may properly be presented here.

Prillieux (6) finds, in his study of the woolly apple aphis (*Schizoneura lanigera* Hausm.) gall on the apple twig, the following facts: No change takes place in the cortical cells pierced by the proboscis of the insect other than the reaction to the mouth parts in them by laying down an "organic sheath" around these mouth parts. The living cells of the wood beneath the mouth parts proliferate strongly, building up a mass of hyperplastic parenchyma in which remnants of ducts are scattered. Multinucleate cells are reported present, but the author does not state in what cells this condition occurs.

Petri (4), in a comprehensive study of the grape Phylloxera root gall, finds the following phenomena to be exhibited: A "warty deposit" of calcium pectate is formed around the setae of the insect after their insertion, by the cytoplasm of the cells in which the setae are present. On the exterior of this sheath Petri believed he demonstrated a layer of tannic substance which upon oxidation gave the characteristic brown color to the old sheaths. The first important changes in the surrounding tissues are the sudden cessation of growth and the non-appearance of differentiation in all near-by cells. These cells show hypertrophy of their nuclei. A short distance from the mouth parts a "ringwall" of hyperplastic tissue springs up.

Rosen (7) studied the grape Phylloxera leaf gall with the following results: The initial depression is produced through "a lessened growth of the attacked mesophyll." "After three to four days of insect attack, the lower half of the leaf tissue which surrounds the portion in which the proboscis is inserted has proliferated enormously. The whole thickness of the leaf in the region immediately around the proboscis shows no proliferation. That portion of the leaf which is beneath the insect does not proliferate but the upper half at the sides of the insect grows upwards and forms the walls of a large insect cavity. Upper epidermal cells and several layers of mesophyll cells in the portion of the gall below the insect, show peculiar thickening and dissolution of their walls." "The investigation establishes the fact that the proboscis may pass through the entire thickness of the leaf." "The continuous sucking action by the insect at one fixed point for fifteen days is believed to be the initial stimulus for gall development."

METHODS

The material of *Pachypsylla mamma* and observational data concerning it were obtained in and near Manhattan, Kansas. That of *P. asteriscus* was acquired in northeastern Texas.

At the critical time in the spring, hatching of the nymphs and gall initiation were observed in the field and laboratory, making possible the fixation of gall material in its earliest stages. All material was fixed in weak chromacetic killing fluid (Schaffner's formula), embedded in paraffin, cut 10 microns thick and stained with Flemming's triple stain. All histological drawings were made with the aid of a camera lucida.

Initial Stages of the Galls

Before attacking the problem of primary cecidium ontogenesis, a very brief statement of the life history of the insect will be given leading up to gall initiation. The following deals with *P. mamma*. *P. asteriscus* has an almost identical life history.

The adults, which are formed immediately after the escape from the galls of the fully grown nymphs in the fall of the year, overwinter in the bark crevices or in ground debris. After mating in early spring, the females lay their eggs on the under side of the young leaves as they begin to protrude from the buds. The point at which the egg is attached is commonly killed, this killing resulting in a prominent hole in the mature leaf. This explains, in part, the presence of numerous perforations in gall-infested leaves. The minute oval eggs (0.3 mm. long) hatch in 2–3 days, the nymph immediately migrating to the upper side of the very young leaf, where, after reaching a position near a principal vein, it settles down to initiate gall development. Once the growing gall has engulfed it, it is a prisoner until its escape the following fall as a mature nymph.

The nymph at the time of gall initiation is a minute, salmon-colored, flattened insect, oval in outline, and measures 0.22 mm. in length. On the ventral side, the setae, which in this minute insect are perfectly formed, extend from the body at a place slightly anterior to the median point. The setal puncturing mechanism measures but little over 1 micron in transverse diameter. There are no special structural modifications in these gall-making nymphs which distinguish them from the large numbers of non-gall-forming Hemipterous larvae.

Under the binocular microscope, the early superficial conditions in gall development may be easily observed and are as follows: The insect's body is pressed close to the upper side of the leaf; a shallow downward evagination forms, lowering the insect into the body of the leaf; when the upper side of the insect has been lowered to the level of the leaf surface, a very rapid upward growth of the leaf tissue surrounding the insect takes place, appearing first like a crater but finally as a closed cone completely covering the nymph. Both *P. mamma* and *P. asteriscus* galls are characterized in their initial stages by this combination of the diverticulum and walled conditions ("Umwallungen" of Küster). The *P. mamma* gall in its further development emphasizes the diverticulum character, the original "cover-cone" developing but slightly, while in the case of *P. asteriscus* no prominent evagination occurs but the cover-cone grows into the prominent, slender, subcylindric process so characteristic of this gall.

Histological Phenomena

At the time when the nymph inserts its setae into the embryo leaf, the leaf cells are not in the primordial condition of undifferentiation but show distinct though partial differentiation; the upper epidermis is very well defined, being composed of large cuboidal cells (fig. 3, at right); the mesophyll shows three distinct layers, and the lower epidermis is set off from the other layers by the minute size of the units composing it. The chloroplasts of the mesophyll cells are well developed as to size and number.

A number of modifications occur coincidently following the insertion of the setal proboscis. These are: (1) The reaction of the cytoplasm of the cells penetrated by the setal structure to this foreign structure, by laying down around it a deposit of organic substance in the form of a very thin, uniform membrane. This structure thus constitutes a definite sheath. (2) A marked hypertrophy of the lower epidermal cells and in a lesser degree of the adjacent mesophyll cells. (3) Hyperplasia sets in in the middle region of the mesophyll surrounding the end of the proboscis. This, however, is greatly restricted. In the case of the *P. mamma* gall (fig. 3), wall formation accompanies the nuclear divisions almost invariably in the early stages; while in the case of *P. asteriscus* wall construction does not occur, the original cells of the mesophyll tiers thus being left almost undisturbed, the cells, however, containing many nuclei. This is best demonstrated in a somewhat later stage (fig. 5*b*). (4) Partial degeneration of the chloroplasts of cells beneath the insect, involving loss of chlorophyll and more or less reduction in size. (5) An increase in the size of the nuclei as compared with those of the normal parts.

The total result of the early hypertrophic and hyperplastic changes is the formation of a saucer-shaped depression in which the nymph (not shown in section) passively lies (fig. 3). This depression or evagination is produced chiefly through the hypertrophy of the elements on the under side of the leaf or that opposite to the insect.

Figure 4 presents a median section of a later stage of the *P. mamma* gall. It will be noted that, compared to the tissue lying at some distance from the larva, the tissue immediately beneath the insect and adjacent to the proboscis shows a marked inhibition of cell-divisional activity. This condition is very characteristic of both galls. These non-dividing cells, however, show the same high protoplasmic content that the actively dividing cells do, so that, when the section is viewed as a whole, there appears to be a zone of meristematic tissue traversing the young gall (shaded cells, fig. 4).

The whole situation, despite the fact of the local growth inhibition mentioned, when contrasted with the condition in a normal partially differentiated leaf, shows that in the early stages of cecidogenesis a process of dedifferentiation is going on, throwing the tissue back into a homogeneous condition. Were events to stop here (fig. 4), we should have a typical kataplasma showing the retrogressive changes of that type of overgrowth. The gall in its further development, however, differentiates into a highly specific prosoplasma, thus furnishing a characteristic example of gall ontogeny recapitulating gall phylogeny.

A still older stage (1 mm. long diameter) of a *P. mamma* gall (median section) is shown in figure 9. The cover-cone has attained almost its maximum size (in the adult gall it is a minute papilla), and the characteristic sub-hemispheric form of the gall has definitely appeared. The occurrence on the very young galls of the large trichomes is worthy of note. These are highly evanescent, never being seen on the adult structure. A presentation of the histology of the nearly mature gall is given in an earlier paper of the writer's (8).

The early histogenesis of a *P. asteriscus* gall is different from that of the *P. mamma* gall. The differences are of course related to the specificity of the galls.

Figure 5 shows the outline of a median section of a very young *P. asteriscus* gall (1 mm. long diameter). At this early stage the fundamental form of the mature gall (fig. 7) is appearing in the prominent cover-cone, the circular outgrowth beneath which in section is exhibited as two lateral processes. The thickness of the region beneath the insect is definitely less than that of the normal leaf as seen to left and right, indicating a marked inhibition of growth in those tissues.

In figure 5*a* are presented the tissue conditions of a critical portion of the section shown in figure 5. Here, as in the case of the *P. mamma* gall, a meristem-like layer is well defined. The details of these two layers and other cellular features are shown in figure 5*b*, which presents the region immediately surrounding the mouth parts. This region, it will be noted, with the exception of a small portion of it directly beneath the sheath-enclosed mouth parts, has maintained the original five-layered condition of the young leaf. Though slightly excessive nuclear division has taken place in the zone of growth inhibition, wall formation has not occurred, the result being the appearance of multinucleate or "giant" cells, the largest of which is that one in which the end of the proboscis terminates. The presence of such a giant cell as the one last mentioned, is also noted in the *P. mamma* galls (fig. 6). Further discussion of this giant cell situation will be given later.

Rosen (7) found an inhibition of growth in the floor of the grape Phylloxera leaf gall but reports no multinucleate condition.

Differing in still another character from the *P. mamma* gall, that produced by *P. asteriscus* shows the very early differentiation of a sclerenchyma layer through the deposition on the walls of the lower hypodermal cells of bands of lignin, simulating the scalariform type of lignification (fig. 5*b*).

The curious condition of the cell in the upper right-hand corner of the section (fig. 5*b*), the cell being almost empty and a rivet-shaped plug of deeply staining matter inserted in the wall, is undoubtedly due to mechanical puncture by one of the two stout bristles with which each leg of the nymph is armed. More injury of this character does not occur probably because of the extreme passivity which characterizes the insect throughout the period of gall formation.

The Sheath

The results of this study are in accordance with Prillieux's (6) and Petri's (4) observations that an organic membrane is deposited by the cytoplasm around the setal proboscis (figs. 5*b*, 6, 8, 10). I found as did Petri in the grape Phylloxera root gall that this membrane is composed of calcium pectate, but was unable as he was to demonstrate a layer of tannic substance on its exterior. In Petri's material the deposit was irregularly laid down or, as he described it, appeared "warty," while in mine it was deposited with remarkable uniformity as to thickness. This sheath is open at its end, thus making possible the direct movement of cellular substances into the end of the setal proboscis. This opening can be demonstrated only with the oil immersion objective in favorable sections (fig. 5*b*), for in many instances the end of the tubular sheath is surrounded by a dense mass of cytoplasmic granules. The nature of this mass I was unable to determine.

Figure 8 illustrates a case in which the insect partially withdrew the setae, then plunged them in again in a different direction. Figure 10 shows an extremely rare condition in which the nymph has withdrawn the mouth parts completely and twice reinserted them. The setae were found as shown, broken off and sticking in the shortest of the three sheaths. The middle sheath is distinctly abnormal. This section shows a condition commonly observed, *viz.*, the projection of the sheath structure beyond the surface of the outer cells.

Examination of half-grown galls and of older ones has shown but one sheath present, indicating that but one insertion of the proboscis takes place, the insect remaining passive and maintaining a stable position for relatively long periods of time. Rosen (7) found the same to be true in the grape Phylloxera leaf gall. Rosen's figure 4 shows a perfect sheath which he inadvertently misinterpreted as "proboscis."

Since in all studies to date of Hemipterous insect cecidiogenesis, an organic sheath has been found surrounding the setal mouth parts, we have reason to believe that the phenomenon is a general one for that group of zoocecidia.

Multinucleate Cells

Multinucleate or giant cells are not unknown in gall tissues, and even are not absent from normal tissues according to Beer and Arber (1), who have found the phenomenon in the stems of 50 dicots and 17 monocots. Prankerd's (5) studies of the occurrence of multinucleate cells in normal tissue are of interest in this connection. He believes they are mostly formed by amitosis. He finds in many instances that a wall is formed later by the two protoplasts resulting from a single amitotic division. Küster (3) presents a number of records of cecidial giant cells in many of which amitosis is reported as the mode of origin of the nuclei. In one instance, the Erineum mite gall on the leaf of the European linden, Küster found, in addition, the degeneration of one nucleus to be a constant phenomenon.

The multinucleate cells found in this study are of much interest, not only because such cells have been rarely reported in the higher galls, but also because a special situation was found in which many of the nuclei were undergoing a process of disintegration.

In the *P. asteriscus* gall, by the time the gall has attained a length of 1 mm., many of the cells, both the one in which the stylets end and certain of those in the immediate neighborhood, show more than one nucleus (fig. 5*b*). The median cell holding the opening of the sheath-enclosed proboscis has generally the largest number, as many as four to six being visible in one focal plane. The other cells possess fewer, more than two being seldom found definitely showing in the same focal plane. Only a few cells in such a section as shown in figure 5*b* are thus seen (oil immersion lens), but these are unmistakably multinucleate.

The nuclei within a particular cell upon critical examination do not appear to be all in the same condition. One commonly, or at the most two of them, may be regarded as normal, while the others exhibit varying stages of disintegration (fig. 5*c*, scale same as fig. 9*a*). In this disintegration process the nuclear membrane disappears, and a prominent vacuole develops in place of a part or all of the nuclear body. The nucleolus persists quite unchanged and may often be found stranded in the cytoplasm near a vacuole.

In the 1 mm. gall of *P. mamma* practically no multinucleate cells are found aside from the large central one in which the mouth parts terminate. But in much older material (the half-grown gall) excellent examples of the giant-cell condition are found. In these, as in the cells of the *P. asteriscus* gall, all stages of disintegration of the nuclei excepting one or two are plainly evident (fig. 9*a*). The problem of the mode of origin of these nuclei is an important one since in some instances, such as the giant cells of the nematode (Heterodera) galls, nuclear proliferation has been reported as amitotic in character. After a most painstaking examination of my slides I am strongly inclined to interpret the situation in terms of amitosis. I am not able, however, to furnish direct positive proof.

The total absence of mitotic figures from the cells of the region concerned might be due merely to the fact that this region is characterized by a marked inhibition of growth as compared to adjacent regions. Individual cells having their origin in the normal embryo leaf before the insect's attack, later, under the inhibiting influence exercised on the three cell layers beneath the nymph, retain their integrity even into the adult gall stage, changing only in the matter of numerical nuclear increase.

This latter change is then followed by degeneration of certain of the nuclei. Since the number of nuclei per cell is always relatively low, seldom if ever going over eight, and since the divisions producing these are scattered over a relatively long period of time (one to four weeks roughly), the chances of catching a nucleus in the act of division are very remote. This of course is true whether the division is of the mitotic or of the amitotic type. For this reason if for no other it is impossible to determine definitely the nature of the nuclear cleavage.

The evidence for amitosis is of an indirect character, such as the frequent occurrence of lobulate nuclei; the very rare display of a constricted nucleus; the frequent occurrence of two nuclei in contiguity; and the very irregular distribution of the nuclei in the cell. But, as heretofore stated, the extremely high infrequency of divisions of any kind makes it quite impossible to determine the nature of the divisions definitely.

Differential Growth between Larva and Gall

Data should be given concerning the difference in time of occurrence of the grand periods of growth of the larva and gall respectively, as this is a matter of more than passing interest. This difference is readily brought out by the following table:

Measurements of larva and gall at different stages of their concomitant development

	Width of larva	Height of gall
Stage 1	0.20 mm.	0.14 mm.
Stage 2	0.22 mm.	0.80 mm.
Stage 3	0.31 mm.	3.55 mm.
Stage 4	0.40 mm.	3.75 mm.
Stage 5	1.69 mm.	5.00 mm.

The grand period of growth for the gall is that of its early existence, while that of the larva comes later when the gall is more than half grown. This inhibition of growth in the larva for an extended period, which is coincident with gall morphogenesis, is in the mind of the writer a very significant phenomenon. Just what it implies in its entirety is quite unknown, but we may feel reasonably sure that it is related in some manner to the production of the gall-making stimulus. The energy of the larva appears at first to be consumed in producing the stimulus, and only when gall development is well on its way to maturity is this energy released for the anabolic processes of the larva itself. This is of course but an assumed generalization which cannot be proved until the problem of the nature of the stimulus involved has been cleared up.

The Stimulus Problem

In many instances the discovery of special structures has been of much value in interpreting function. When we raise the physiological problems of the nature of the gall-forming stimulus in these prosoplasmas and look for some hints concerning it from structural conditions, it must be confessed that none are given. I cannot find a single structural fact produced by any one in the study of the initial stages of Hemiptera galls, that throws any light whatever upon the profound problem of the nature of the highly specific stimulus applied by the insect to the embryonic plant tissue.

Rosen's (7) "belief" that "the continuous sucking action by the insect at one fixed point for fifteen days is the initial stimulus for gall development" will not hold, for there are too many non-gall-making hemipterous insects sucking at one place for extended periods without ensuing hypertrophy or hyperplasia. Further, as previously shown, little or no ingestion takes place, for in the early days of its gall-making activity no increase in size of the larva occurs. It is hardly conceivable that the pumping action of the insect's sucking apparatus would function without ingestion going on, especially when the proboscis end was in the presence of liquid or semi-liquid food. Attention may also be called to the fact that no experimental evidence along this line has yet been produced.

On *a priori* grounds it would seem much more natural to assume with Küster that such prosoplasmas were "*Chemomorphosen*"; that a highly specific chemical substance was introduced producing the radial effects noted. This conception, however, has not the slightest direct evidence for its support, since such a specific chemical substance producing a prosoplasma has never been demonstrated. The difficulty of demonstrating it may be of course due only to the exceedingly minute amounts of it which are formed. Conservatism here as elsewhere is the desirable position to assume.

One who has repeatedly removed these minute, delicate gall-inciting organisms from the plastic, watery tissue of the embryonic galls is impressed with the possibility of interpreting the situation in terms of correlation phenomena. The writer ventures to suggest that the insect may be acting as a whole in the matter.

Though the structural facts concerning gall initiation and early development are of much interest, we must confess that these data give us no more fundamental, explanatory information concerning gall ontogenesis than do the structural facts of normal growth explain normal ontogenesis. We must remain satisfied with having merely demonstrated the leading morphological facts involved in the early development of these two Pachypsylla galls.

SUMMARY

1. The early stages of two hemipterous insect galls of the hackberry leaf were studied. The insects belong to the genus Pachypsylla of the Psyllidae.

2. The newly hatched nymphs after placing themselves upon the upper side of the young leaf initiate the following concomitant changes: (*a*) Formation by the cytoplasm of the affected cells of a sheath-like structure around the inserted setal proboscis. The cells are not killed. The pro-

boscis is inserted but once, the larva retaining its position through the period of gall development. (*b*) Hypertrophy of the epidermal and adjacent mesophyll cells on the lower side of the leaf opposite to that attacked by the insect. (*c*) A slight hyperplasia generally appears in the cells immediately surrounding the end of the mouth parts. (*d*) Degeneration of chloroplasts in a zone beneath the larva. (*e*) An increase in the size of the nuclei in the same zone.

3. The above noted changes (*b* and *c*) produce a downward evagination which lowers the insect into the body of the leaf, after which process a "cover-cone" rapidly grows over it, springing from the tissue surrounding the larva.

4. Multinucleate cells appear in the tissue of the floor of the larval chamber, most of the nuclei, however, soon disintegrating. The mode of origin of these nuclei is believed to be amitotic, though this under the condition of high divisional infrequency cannot be definitely proved.

5. The very early differentiation of a sclerenchyma layer, one cell in thickness, is highly characteristic of one of the galls.

6. The nymph shows practically no increase in size until the gall is about half developed. Thus the grand period of growth in the gall is at the beginning of its ontogeny while that of the insect is postponed until cecidiogenesis is well under way.

7. The morphological facts discovered furnish no assistance whatever in the formulation of a theory concerning the nature of the stimulus used by the larval insects in initiating and carrying to completion the highly specific gall structures characteristic of the two species respectively.

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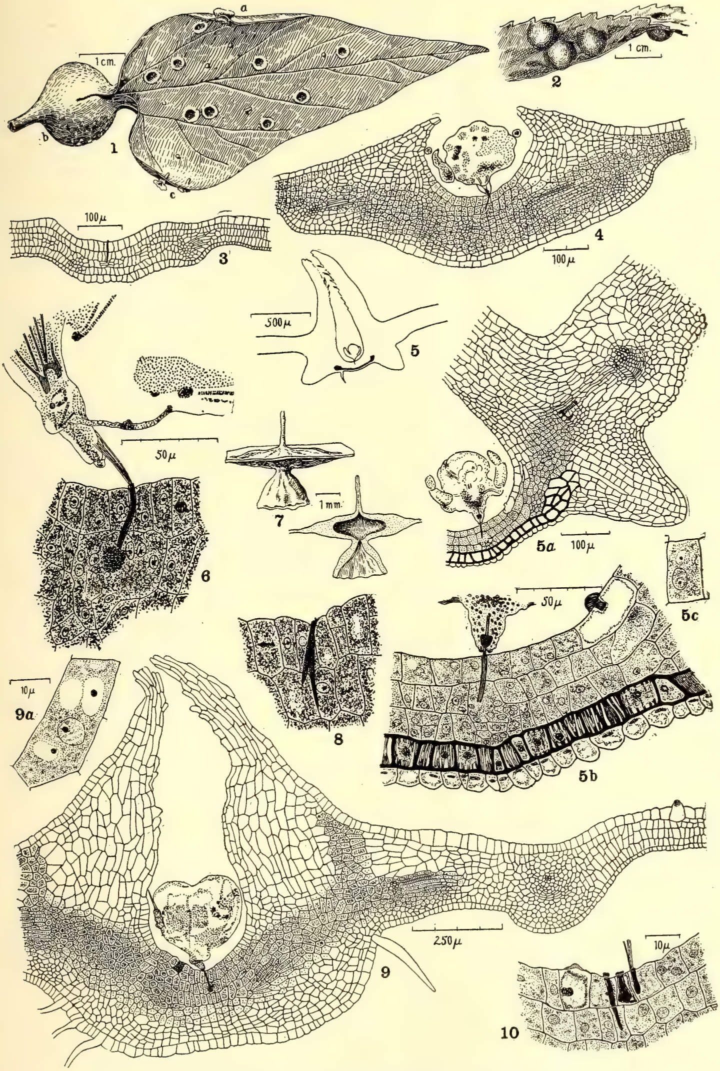
a
1cm.
b
1
c
2
1 cm.
3
100μ
4
100μ
500μ
5
50μ
6
7
1mm.
5a
100μ
5c
50μ
5b
8
10μ
9a
250μ
9
10μ
10

WELLS: EARLY STAGES IN THE DEVELOPMENT OF PACHYPSYLLA GALLS.

EXPLANATION OF PLATE XVIII

FIG. 1. Leaf of *Celtis mississippiensis* showing three distinct types of Pachypsylla galls: (*a*) Variety of *P. mamma*. (*b*) Compound gall of *P. venusta*. (*c*) Gall of *P. asteriscus*.

FIG. 2. Galls of *P. mamma* (typical form) on *Celtis occidentalis* leaf.

FIG. 3. Earliest initial stage of the *P. mamma* gall.

FIG. 4. Early stage of *P. mamma* gall.

FIG. 5. Outline of longitudinal section of early stage of gall of *P. asteriscus* (1 mm.).

FIG. 5*a*. Tissue study of lower right-hand portion of section shown in figure 5.

FIG. 5*b*. Cell study of region around mouth parts shown in figure 5*a*.

FIG. 5*c*. Multinucleate cell from same gall as figure 5 showing two nuclei, one of which has partially disintegrated.

FIG. 6. Detail from section of very young *P. mamma* gall; setae partially withdrawn from sheath.

FIG. 7. Gall of *P. asteriscus*, external and internal views.

FIG. 8. Detail from section of very young *P. mamma* gall showing the forked sheath formed through withdrawal and reinsertion of the setae.

FIG. 9. Section of very young *P. mamma* gall (1 mm.).

FIG. 9*a*. Cell from half grown *P. mamma* gall showing multinucleate condition. Note nuclei which show a degenerate condition.

FIG. 10. Detail from section of very young *P. asteriscus* gall showing three sheaths (more than one is extremely rare); setae still inserted in one to right.

THE UPWARD TRANSLOCATION OF FOODS IN WOODY PLANTS. II. IS THERE NORMALLY AN UPWARD TRANSFER OF STORAGE FOODS FROM THE ROOTS OR TRUNK TO THE GROWING SHOOTS?

Otis F. Curtis

There is apparently a very common belief that in most trees considerable quantities of the carbohydrates, that have been stored in the lower trunk and in the roots, move up as growth starts in the spring and are used in shoot and leaf formation.

The arguments which seem most commonly to be put forward as proof of such an upward transfer are that quantities of food are stored in the xylem tissues; that these are present in soluble form in the water-conducting vessels at the time spring growth commences; and that these foods rapidly disappear at about the time of this rapid shoot development. From these facts it would seem reasonable to think that the food had moved up with the water to the growing shoots, but, as shown in a recent paper (Curtis, 1920), the mere presence of soluble foods in water-conducting tissues cannot be considered as proof that the foods move with the water. In fact, it was shown that there is no appreciable longitudinal transfer of soluble foods through the xylem.

Some ringing experiments of Hartig's (1858) have also been considered as proof of the movement of foods from the roots to the growing shoots. At intervals of eight days from the first of April, 1857, until the middle of September of the same year, he ringed young oak trees of about the diameter of one's arm. The rings were two inches broad and were situated four feet from the ground. Some trees were also cut down at the time of ringing, but he does not state whether these were cut early or late in the season. Observations made in the spring of 1858 showed that all trees ringed previous to June 30, 1857, had lost the starch from below the rings, while those ringed after June 30 contained starch. The starch from these also, however, had disappeared by the fall of 1858. As the starch had not disappeared from some of the roots of the felled trees, he concluded that the food stored in the roots normally moves up with the water through the xylem and is used in shoot growth.

As has been previously shown, no appreciable quantities of food move longitudinally through the xylem and it seems very probable that the food below the rings disappeared because it was used in root growth and in diameter growth of the trunk. The only point tending to contradict this is that in some of the felled trees the starch did not disappear. This lack of removal from the stumps of felled trees, however, may have been due to an excess of water following removal of the transpiring surface and resulting in a check on respiration or in death. When the stumps were healthy, as indicated by the development of shoots, the starch did disappear. Hartig failed to state the time at which trees were felled as well as the relative number of stumps which retained or lost their food stores.

The fact that the diameter growth of a trunk was very much decreased below a ring is additional proof that there is no large excess of food stored in the roots. Hartig explained this weak growth below a ring as due to the inability of the food, which he considered as moving up through the xylem, to move radially to the cambium. He believed that only that food coming through the phloem could be used in cambial growth.

Other data which have been considered as proof of the use of food from the roots for spring shoot growth have been presented by Leclerc du Sablon (1906) who determined the effects of ringing at different seasons on the amounts of carbohydrates found in roots and stems of a number of woody plants. He concluded that, as a general rule to which there may be exceptions, the roots of woody plants act as storage organs from which the carbohydrates move up in the spring. The data he offered, however, are far from conclusive. Some results he obtained in ringing experiments on the pear are presented in table 1.

The analyses for April 13 alone suggest that upward translocation from the roots might have taken place in the spring and that the ring has prevented this upward transfer, for in that tree ringed February 9 the roots have a higher content than the check and the stems a lower content. But in a preliminary series (in 1904) he found similar differences between individuals taken at one time. For the quince, samples taken from four different plants on March 17 showed a maximum difference in carbohydrate content per 100 grams of dry material of 5.7 grams for roots and 4.9 grams for stems. With the pear, the corresponding differences were respectively 2.7 and 1.4. It is true that trees differing in external characteristics were definitely chosen for these samples, but similar differences might easily

TABLE 1. *Data from Leclerc du Sablon to show effect of ringing on distribution of food between roots and stems of pear trees. Total carbohydrates expressed as percentage of dry weight.*

Date at which Sample Taken	Not Ringed		Ringed Feb. 9		Ringed May 8	
	Roots	Stems	Roots	Stems	Roots	Stems
Feb. 18	30.3	23.0	——	——	——	——
Apr. 13	22.4	21.3	25.6	18.3	——	——
June 16	27.9	23.7	27.9	29.5	17.5	29.0
Aug. 4	29.2	24.7	26.5	33.2	18.3	27.0
Sept. 24	33.8	25.7	19.3	29.1	21.4	29.5
Dec. 1	29.3	25.4	17.4	25.9	17.5	25.8

have occurred between the other trees. Furthermore, the increases in carbohydrate content of the roots of ringed plants over that content found earlier in the season, shown on June 16 for those ringed on February 9 and as shown on June 16 and August 4 for those ringed May 8, would be hard to explain except as resulting from individual variations or from the healing of the wounds. In the quince, an analysis on April 13 showed greater carbohydrate content in the roots of the ringed tree than in those of the tree not ringed, but the stem of the ringed tree also showed a carbohydrate content greater than the stem of the check. Evidently the whole tree had a higher carbohydrate content.

Hartig, Leclerc du Sablon, Butler (1917), and others have shown that before growth starts in the spring the roots may contain a higher percentage of carbohydrates than the stems, but the stems have more supporting tissue and the percentage composition may therefore mean nothing unless the total mass is known. The actual amount of carbohydrates in the roots may be less than that in the tops, even though the percentage composition is high.

Data showing that the mass of carbohydrates stored in the roots is actually much less than that stored in the tops have been presented by Chandler (1917) who has calculated, from percentage concentrations obtained by Butler, the relative amounts of food available in the roots and tops of an apple tree. His data are presented in table 2.

TABLE 2. *Approximate amounts of dry matter, starch, and saccharose at the time buds are swelling, in case of a seven-year-old Bismarck apple tree that has been growing in sod.*

Part of Tree	Actual Dry Weights, Pounds	Pounds of Starch Calculated	Pounds of Saccharose Calculated
1-yr. twigs	3.15	0.98	0.12
Older branches	21.00	6.72	0.17
Trunk	15.13	5.14	0.11
Totals for parts above ground	39.28	12.84	0.40
Large roots	14.15	5.43	0.28
Small roots	6.49	2.37	0.06
Totals for roots	20.64	7.80	0.34

These figures are, of course, only suggestive, as the trees analyzed and the one weighed were grown under different conditions. But the error would tend to be in favor of large root storage, for the tree weighed had been grown in sod under conditions favorable to larger root growth. In this instance the roots weighed 52.5 percent as much as the tops. Pickering (1917) gives data showing the relative weights of tops and roots of a number of trees varying from 10 to 20 years old. The average root weight of 461 apple trees was 22.9 percent of the tops, that of 15 pears was 23.5 percent, that of 6 Damsons was 25.2 percent and that of 44 plums was 28.3 percent.

The relative root and top weights would, of course, vary with the soil and the climate, but there seem to be good indications that tree roots may not greatly exceed 50 percent of the top weight. Therefore, though the roots may have a carbohydrate content greater than the tops when measured as percentage of dry weight, the total quantity of carbohydrates in the roots is much less than that in the tops, and, since the roots must need quantities of food for their own use, it seems doubtful whether any is normally carried to the tops for shoot growth. The indications that root growth commences in the spring before shoot growth, as discussed later in this paper, may be considered as further proof that the food stored in the roots is used primarily by the roots.

Data obtained from experiments designed to determine the path of upward translocation, a subject reported in a recent paper by the writer (Curtis, 1920), offer evidence that little or none of the food stored in the trunks or roots of trees is normally moved up to be used by the developing shoots and leaves.

In one group of experiments, large numbers of twigs and branches were ringed early in the spring while the buds were still dormant or were just beginning growth. These rings were made at different distances from the tip in order to determine from how far back food was withdrawn for shoot growth.

Since, as was shown in the previous paper, no appreciable upward movement of foods occurs through the xylem, the growth of a shoot above a ring would serve as an approximate measure of the amount of food available. If the ring were back far enough from the growing tip to allow for growth practically as great as that on unringed twigs, it would seem that these twigs need not draw on the food stored at greater distances.

A large number of stems of *Acer saccharum* were ringed on April 5 at different distances from the tips. In one series the rings were in the first-year wood, in another in the second- or third-year wood, and in another the rings were in that part of the stem ranging from five to fifteen years old. Some of the stems had made terminal growths in the previous year of from only 1 to 10 centimeters, while others had made growths of from 20 to 40 centimeters. In each case a check stem was chosen as nearly matching the ringed one as possible. The check and ringed stems were usually the two terminals of a pair produced by dichotomous branching. Such a variety of branches was used that no attempt will be made to give more than a brief summary of the results.

Of 15 twigs ringed in the one-year-old wood, the average terminal growth on May 6 was 0.84 cm. That of the corresponding stems not ringed was 2.22 cm. Of those ringed in the two- and three-year-old wood the average terminal growth was 2.03 cm., while that of the corresponding checks was 2.25 cm. The leaves of the ringed stems in these cases did not show the bronze tinges that were common in the normal young leaves, but were a bright green. At the same date, May 6, there were no apparent differences in the growths of stems not ringed and of those ringed back on the 5- to 15-year-old stems. On May 25 measurements were made of these stems. An average of the shoots of ten stems of this series showed a growth of 9.96 cm., while that of the corresponding check stems was 11.09 cm. The older stems, whether the diameter was large or small, showed growth fully as great as that of those not ringed, but some of the younger stems showed somewhat lessened growth which lowered the average for the growth of ringed stems. In most cases growth had ceased and terminal buds were beginning to develop at the time of measuring.

On April 7, a number of stems of a pear tree growing in sod were ringed. These stems ringed in the one- and two-year-old wood showed distinctly lessened shoot growth, but those ringed where the diameter was from 1.5 to 3 cm. showed growth fully as great as that of the unringed stems.

On May 16, 1919, stems of an apple tree that was just beginning growth were ringed just below the base of the one-, two-, and five-year-old wood. At the time of ringing, the lengths of the shoots measured to the tips of the infolded leaves were from 1.5 to 2.0 centimeters. At the same time a single branch was ringed at its base where it measured 3.8 centimeters in diameter. The diameter of the main trunk just below the lower limbs was 11.0 centimeters. All the stems ringed in the fifth-year wood (group 4) were less than one centimeter in diameter at this point, with the exception of those lettered *c* and *j* which were respectively 1.2 and 1.5 centimeters in diameter. The growth was completed in most of the twigs when the measurements were taken on June 15. These data are recorded in table 3.

TABLE 3. *Pyrus malus. Ringed May 16, 1919. Measurements taken June 16. All of the same letter excepting in column 5 were closely matched. Column 5 and letters a to h on one tree, i to n on another. The data are represented as growth in centimeters.*

	1 Not Ringed	2 Ringed Below Base of One-year-old Wood	3 Ringed Below Base of Two-year-old Wood	4 Ringed Below Base of Five-year-old Wood	5 Ringed at Base of Branch 3–8 Cm. in Diameter
a.....	17.5	1.0	4.5	11.5	12.5
b.....	14.5	0.7	.5	5.0	13.0
c.....	9.5	0.4	3.0	10.0	13.0
d.....	15.5	0.4	2.0	9.0	12.5
e.....	17.0	*3.0 wound healed	6.0	21.0	10.5
f.....	16.0	1.0	—	11.5	
g.....	10.5	*5.5 wound healed	3.5	10.5	
h.....	10.0	2.5	*1.5 broken	—	
i.....	14.5	*2.5 wound healed	6.5	10.0	
j.....	19.5	0.4	4.5	13.5	
k.....	17.5	1.5	6.0	—	
l.....	18.5	1.8	7.0	—	
m....	17.0	*6.5 wound healed	3.5	—	
n.....	14.5	*3.0 wound healed	4.0	9.5	
Ave. .	15.14	1.08	4.42	11.15	12.3

* Not included in average.

From the table it seems that shoot growth is fairly vigorous when no food further back than that obtained from a branch about one centimeter in diameter is available.

A somewhat similar experiment was tried with *Fagus grandifolia.* In this case the ringing was done before the buds had started. The data are reported in table 4.

TABLE 4. *Fagus grandifolia. April 7 to May 24.*

	Ave. Length of Shoot in Mm.	Ave. Number of Leaves
Twigs not ringed	186.4	6.6
Ringed in the middle of the one-year-old wood	21.4	2.1
Ringed at the base of the one-year-old wood	42.8	3.3
Ringed in the wood three to five years old which in all cases was less than one centimeter in diameter	59.1	5.0

These data as well as those reported in a previous paper (Curtis, 1920, tables 8–10) indicate that, when the ring is no further back than the 5- to 10-year-old wood, the growth of the shoots above the ring approaches more and more nearly that of the unringed stems. It will be necessary to use larger numbers of branches before one can attempt to state the distance from which food may be withdrawn.

Even if one could use large numbers of uniform stems that have been grown under uniform conditions, it would be difficult to determine from ringing experiments alone as to the exact distance of upward movement, for a check in growth may result not from lack of food but from lack of water due to the fact that no new xylem would be formed in the region of the ring, because in some trees much of the water may be carried through this new xylem. It is to be noted that, in practically every case in which the wound was not well protected by a coating of paraffin, the growth was distinctly decreased as a result of a deficiency of water due to drying of the xylem.

Not only is there considerable food stored in the twigs and young branches which becomes available for shoot growth in the spring, but the food manufactured by the new leaves soon after they open also becomes available for continued shoot growth.

The data reported in table 5 indicate that, soon after the shoots have started, much or all of the food necessary for continuing growth is produced by the leaves of that same shoot.

In the experiments with apple and in the first of the experiments with Ligustrum, the growth of the ringed twigs with leaves is fully as great as that of the twigs not ringed. In fact, in these two cases the data indicate that ringing has even increased growth above that in the checks. This may be because the ring has increased the food supply by preventing removal of that produced by the new leaves. In the other experiments, with the exception of the 1918 experiment with Philadelphus, the growth of the ringed stems which retain their leaves is nearly as great as that of the normal stems. Even the results of the 1918 experiment with Philadelphus are not in opposition to the hypothesis that a large part of the food used for continued growth of a stem is produced by the leaves of that stem, for, as indicated in table 5 and also in the more detailed tables 1 and 2 of the previous paper, only about one third of the current year's growth was above the ring in the 1918 experiment and this part carried only the younger leaves, while in the 1919 experiment the entire new shoot with all its leaves was above the ring. The fact that the stems which were defoliated for a distance of from 15 to 20 centimeters from the tips in 1918 (group 3) showed such good growth, while the shoots of the 1919 experiment (group 3) showed such poor growth gives additional proof that the leaves of the new shoot supply a large part of the food used in growth after a few leaves have once opened. In 1918 that food was available which was produced by the many leaves on the lower non-defoliated part of the stem, while in 1919 only stored food was available as the entire new shoot was defoliated.

TABLE 5. *Experiment to determine how much food used in shoot growth may be produced by the leaves of that shoot.*

	1 Not Ringed, Leaves Remaining		2 Ringed, Leaves Remaining		3 Not Ringed, Leaves Removed		4 Ringed, Leaves Removed	
	Original Length of Shoot	Gain in Cm.	Original Length Above Ring, Cm.	Gain in Cm.	Original Length of Part Defoliated	Gain in Cm.	Original Length Above Ring	Gain in Cm.
Apple. June 11 to June 30. Ave. of 6 stems	25.0	4.48	15.0	5.3	15.0	3.37	15.0	0.25
Ligustrum ovalifolium. June 18 to July 3. Ave. of 7 stems		13.61	21.7	14.61	22.0	7.41	21.7	0.71
Ligustrum ovalifolium. June 19 to July 3. Ave. of 6 stems		12.41	20.8	10.58	20.9	5.75	21.1	0.23
Ligustrum ovalifolium. July 6 to July 22. Ave. of 6 stems		12.23	11.8	7.2	10.9	7.62	11.8	0.28
Ligustrum ovalifolium. July 11 to July 22. Ave. of 25 stems		9.94	17.8	8.19				
Philadelphus pubescens. May 30 to June 8, 1918. Rings in new growth. Ave. of 5 stems	54.6	34.1	17.0	11.75	17.0	27.0	17.0	0.5
Philadelphus pubescens. May 30 to June 4, 1919. Rings in old wood below base of new growth Ave. of 14 stems	18.8	16.36	21.1	12.96	17.4	7.96	18.5	1.16
Philadelphus pubescens. June 3 to June 6, 1919. Rings in old wood below base of new growth. Ave. of 8 stems	37.6	9.38	38.4	7.13	40.0	3.56	30.8	1.44

Discussion

The available data are not sufficiently extensive to enable one to conclude from how far back food is withdrawn to be used in shoot growth. It is probable that the amount of upward movement depends upon the kind of tree, its age, and conditions of previous growth, as well as on conditions during the current season.

Leclerc du Sablon suggested that some trees may store but little of their food in the roots, while others store quantities there to be used later in shoot growth, but his experiments supposedly proving the latter condition are far from convincing. It is to be noted that he used young trees only three to four years old, and, though his data offer no conclusive proof, it is possible that such young trees might show more upward transfer of foods; but it is just as possible that, when a tree becomes well established, there is normally very little upward translocation. Other conditions being equal, one would expect little or no withdrawal of carbohydrates from below if during the spring growing season there were a deficiency of water and perhaps of mineral nutrients, especially nitrates, and the days were bright. Under such conditions vegetative growth would tend to become checked early, and the new leaves would soon begin to accumulate carbohydrates through photosynthesis.

If root growth commenced in the spring before shoot growth, or even if growth began in both at about the same time, one would expect that most of the food present in the roots would be immediately needed by the roots. No very conclusive evidence on this point is available, but Goff (1898) found that root growth may commonly precede the swelling of buds. Observations were made by digging trenches early in the spring and measuring the amount of new growth that had occurred up to the time of digging. Such early root growth was found to occur in the following plants: *Acer saccharum*, *Pyrus malus*, *Pyrus communis*, *Prunus cerasus*, *Prunus virginiana*, *Betula alba*, *Morus alba*, *Cornus stolonifera*, *Eleagnus hortensis* var. *Songorica*, *Ribes rubrum*, *Ribes nigrum*, *Ribes oxyacanthoides;* as well as in nine species of gymnosperms and a few herbaceous perennials. There were only two possible exceptions recorded.

Furthermore, data presented by Jones (1903) would indicate that root growth precedes stem growth. It was found that the water content of the trunk of the sugar maple increased from 31.5 percent in December to 36.5 percent in March, while from March 15 to April 28 the water content increased to 47 per cent. After this date the buds opened and the water content fell off. This rapid increase in water content just previous to the opening of the buds, which occurs not only in the maple but in all the other deciduous trees examined, though it cannot be considered as conclusive proof, yet at least suggests that the absorbing organs, the roots, have started growth early, making possible a rapid absorption of water just previous to shoot growth.

The data obtained from ringing dormant stems show that, when the ring is no further back from the growing tip than that part of the branch from 5 to 15 years old or from one to five centimeters in diameter, the growth may be practically as extensive as when no ring is made. When the growth was somewhat lessened by ringing, it may have been due, not to a lack of food, but to a deficiency of water, as the ring, of course, prevented the formation of a new layer of xylem. Furthermore, although the rate of starch loss was more rapid when the ring was near the tip, indicating more complete usage of stored food, yet the rate of starch loss when the ring was in the older wood was approximately the same as from a normal stem. These results indicate that normally very little food is withdrawn and carried up from the main trunk or roots to be used in shoot growth. It would seem therefore that, especially in older trees, the food in the branches is more than sufficient to initiate shoot growth, and that, since much of the food necessary for continued growth may be produced by the young leaves, there may be no tendency to draw upon that food stored in the roots. Furthermore, the fact that a cutting no longer than six inches may produce a short shoot with leaves and also a callus and roots, when no food can be obtained from storage organs outside that small bit of stem, would indicate that a shoot on a large branch need not draw on food stored at great distances, as from the trunk or roots.

There seems to be little foundation for the statement (Butler, 1917) that the carbohydrate stored in the young root tip is digested in the spring and carried up the trunk to the stems for shoot formation. It seems more probable that the roots themselves use nearly all, if not quite all, of the foods stored in them. When one considers that root growth probably commences earlier in the spring than shoot growth and may also continue later in the fall; that at no time can roots produce their own foods, as can the shoots as soon as a few leaves form; and that the tree roots probably store a smaller mass of food than do the tops, it is then difficult to see how food from the roots can be of very great aid in the shoot formation of a tree.

SUMMARY

Facts commonly considered as proving that the food which is stored in the roots and lower trunks of many trees is carried up to be used in shoot formation cannot be considered as actually proving such upward movement.

When a ring is made on that part of a stem from 5 to 15 or more years old or from one to four or more centimeters in diameter, the growth above the ring approximates that of a normal stem, which fact indicates that upward movement of food from points below the ring is not essential.

When shoot growth is well started, much of the food used for continued growth may be produced by the leaves of that shoot, which fact indicates that considerable growth may take place when but little stored food is available.

The data at hand are not adequate to settle the question as to how far back from the tip food is withdrawn to be used in shoot growth. In fact, the amount of removal probably varies with different species and with different individuals, depending on the conditions of the current as well as of previous seasons. It seems very probable, however, that there is normally no upward movement of foods from the roots and perhaps little or none from the main trunk.

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EARLY STAGES IN THE DEVELOPMENT OF THE SPOROPHYTE OF SPHAGNUM SUBSECUNDUM

Geo. S. Bryan

In the spring of 1913 while following through the life cycle of Sphagnum in the field the writer was able to find a great wealth of young sporophytic material. In order to make a detailed study several strengths and combinations of chromic and acetic acids were used as fixing agents. However, it was extremely disappointing to find that in every case the young sporophytes were completely plasmolyzed so that an interpretation of sectioned paraffin material was impossible. Time now being an important factor, as a last resort the dissection of the young sporophytes from the venters of the archegonia was undertaken. While the task was a laborious one, it proved to be relatively simple. The archegonia were first dissected from the tips of the branches on which they were growing. Then the basal portion of each venter, or, in the older stages, of the swollen stalk was carefully dissected, using slender needles for the operation. After the venter or the swollen stalk had been opened, gentle pressure on the neck of the archegonium was usually sufficient to cause the young sporophyte to slip out.

More than one hundred young sporophytes were examined. The study and drawings were made from the living material and are illustrative of the general conditions found. While the results confirm in a general way the work of Waldner, there are points of difference which make the publication of these observations seem worth while.

HISTORICAL

In 1858 Schimper[1] first attempted to trace the development of the sporophyte of Sphagnum. He described the first wall as being almost horizontal; then quickly follow radial, vertical, and horizontal walls, so that in a short time the single cell has become a long, many-celled, pear-shaped body. Schimper further thought that only the lower part of the young sporophyte—that which bores its way into the stalk of the archegonium—develops into the mature capsule. The upper portion he believed disintegrated and was resorbed.

In 1887 Waldner[2] studied the details of the development of the sporophyte using chiefly *Sphagnum acutifolium* Ehrh. He states that the egg in the venter of an archegonium ready for fertilization is ovoid or somewhat pear-shaped, and shows clearly a nucleolus with a nuclear body (his Taf. II, fig. 1). The fertilized egg is drawn very indefinitely, and no description is

---

[1] Schimper, W. P. Versuch einer Entwickelungsgeschichte der Torfmoose. Pp. 96, pls. 1–27. Stuttgart, 1858.

[2] Waldner, M. Die Entwickelung der Sporogone von Andreaea und Sphagnum. Pp. 25, pls. 1–4. Leipzig, 1887.

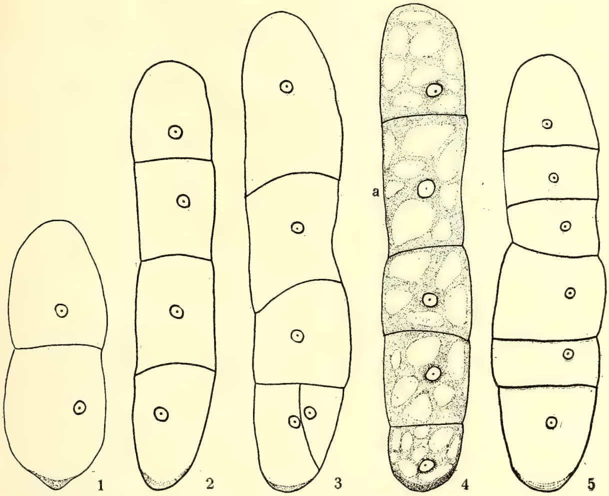


FIG. 1. Embryo of 2 cells. FIG. 2. Embryo of 4 cells. FIG. 3. Embryo of 4 primary segments. Apical cell much elongated, probably preparatory to division. Basal segment has divided irregularly. FIG. 4. Embryo of 5 cells, showing cytoplasmic details at this stage. FIG. 5. Embryo of 6 cells. All × 300.

given save that it is surrounded by a hyaline mass of slime (Taf. II, fig. 2). The fertilized egg divides transversely, the upper half being the apical cell. A two-celled embryo is drawn in which the upper cell is relatively small as compared with the basal segment. Waldner states that the apical cell, by walls parallel to its base, cuts off a series of segments from 6 to 8 in number. The basal cell makes only a few irregular divisions and does nothing more. Waldner's figures show that the wall separating the basal and apical segments is sharply defined and may be followed for some time in the subsequent stages of development of the young sporophyte.

FERTILIZATION, AND THE DEVELOPMENT OF THE YOUNG SPOROPHYTE

After a number of attempts, fertilization was brought about in the laboratory by squeezing the heads of antheridial plants and immediately allowing the exuding liquid to drop on the tips of archegonial plants which were almost entirely submerged in water. A detailed account of these experiments may be published at some later date. Unfortunately, at the time of fertilization there occurs in the venter of Sphagnum the development of a considerable amount of mucilaginous matter which entirely invests the fertilized egg, and which thus far has proven difficult to penetrate

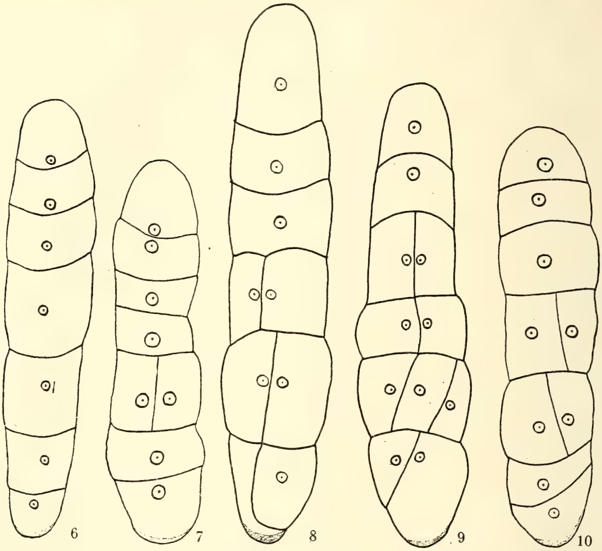


FIG. 6. Embryo of 7 cells. Slender type. FIG. 7. Embryo of 7 primary segments. First vertical wall appears in one segment. FIG. 8. Embryo of 6 primary segments three of which show vertical walls. The two cells of the basal segment are rounding away from each other. FIG. 9. Embryo of 6 primary segments, the basal portion much larger than the apical. FIG. 10. Embryo of 7 primary segments. All × 300.

with fixing agents. Since the results are not entirely satisfactory, because of plasmolysis, statements concerning the structure of the fertilized egg will be omitted.

The first wall is at right angles to the axis of the archegonium and divides the young sporophyte into two approximately equal cells which elongate in the direction of the axis of the archegonium (fig. 1). The basal cell is characterized at its lower extremity by a peculiar greenish zone which is probably related in some fashion to the digesting action of this cell. This zone is clearly visible in all the early stages (figs. 1–15) and serves as a convenient means of distinguishing the basal from the apical end of the embryo when dissected from the venter of the archegonium. After the two-celled stage has been reached there are further divisions by walls parallel to that first formed, resulting in the production of a filament of cells usually six or seven in number before walls make their appearance in other planes (figs. 1–6). Occasionally, as illustrated by figure 3, the basal cell may divide quite early in other than a transverse plane. However, the filament of cells without such irregular divisions is the common occurrence in the material studied at these early stages.

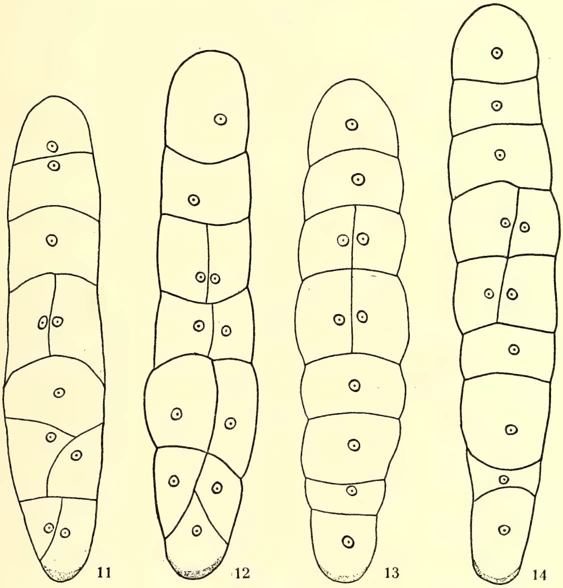
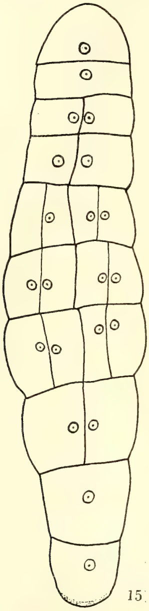


FIG. 11. Embryo of primary segments. Two basal segments show irregular divisions. FIG. 12. Embryo of 6 primary segments. Basal segment irregularly divided. FIGS. 13 and 14. Slender types. No irregular divisions in basal segments. All × 300.

In the later stages of development the writer has been unable to trace with any degree of certainty the original wall separating the basal and apical cells which Waldner in his drawings shows as sharply defined. As illustrated by figures 2–14, it is evident that in the material here studied there is no sharp delimitation, hence an exact statement as to the part contributed by each of these cells would be unwarranted. While no division figures could be found, the elongation of the apical cell as shown in figures 3, 8, and 12 furnishes strong evidence of apical growth, and finds frequent corroboration in the position of the nuclei as illustrated by the two uppermost cells in each of figures 7 and 11. Whether this apical growth may be supplemented by the occasional intercalary division of a primary segment is questionable. Cell *a* in figure 4 suggests such a possibility. The elongation of this cell together with the size of the nucleus makes it seem probable that such a division is about to take place.

While it is impossible to trace with absolute certainty all of the divisions undergone by the basal cell, a study of basal regions shows two distinct types of divisions (figs. 2–21). As illustrated by figures 3, 8, 9, 11, and 12, the basal cell divides irregularly, while in the embryos shown in figures 6, 7, 13, 14, and 15, if the basal cell has divided the divisions are regular, *i.e.*, brought about by walls parallel to that first formed. This irregularity or regularity expresses itself later in what might be termed two types of young sporophytes. The first of these, in which the lower portion is distinctly bulbous, is derived from the irregular type. The cells formed by these irregular divisions grow considerably in size and tend to round away from each other (figs. 16–17). Such embryos stand in sharp contrast to those of



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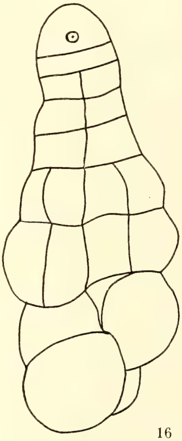

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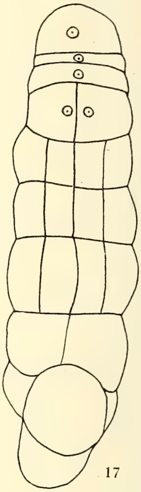

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FIG. 15. Embryo showing 10 primary segments. Basal portion of sporophyte slender and without irregular divisions. FIG. 16. Bulbous type of young embryo. Cells of basal portion have strong tendency to round away from each other. FIG. 17. Embryo showing probably 9 primary segments. Basal portion bulbous. All × 300.

the second type in which the basal cells are regular and slender and remain so for some time, apparently serving as a distinct boring organ aiding the young sporophyte in digesting its way into the stalk of the archegonium (figs. 15, 18, 20, 21). Such a structure is somewhat suggestive of an inverted suspensor. Figures 22 and 23, sketched from living material, give some idea of how quickly the young sporophyte bores its way out of the venter. In figure 22*a*, the embryo has already begun to digest the cells of of the venter immediately below it. In figure 22*b*, the embryo has worked its way entirely out of the venter and is embedded in the stalk of the archegonium. Figure 23 shows a still later stage in which the stalk of the archegonium has developed considerably in thickness, and illustrates the expansion of the basal portion of the young sporophyte to form the foot. It should be noted, however, that the immediate basal cells of the slender type do not form this expanded foot. In the embryo shown in figure 20, the foot will arise from the cells above the segment $x - x$. In figure 21 the expansion to form the foot proper can be observed at $b - b$.

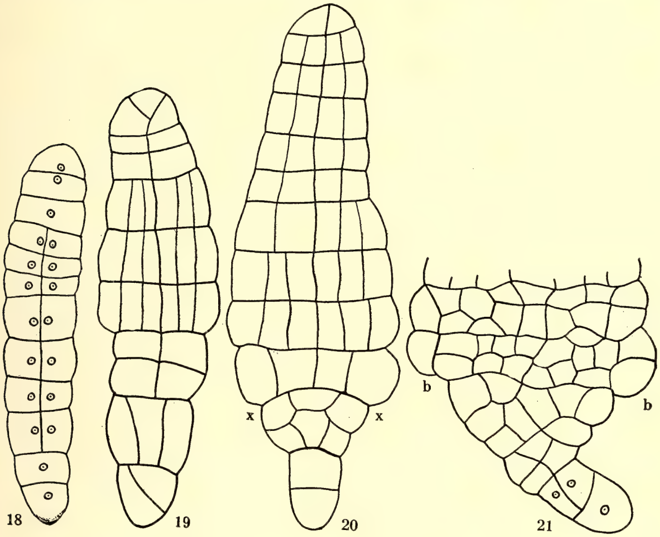
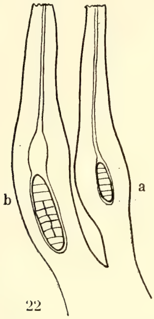


FIG. 18. Embryo showing 12 primary segments, the maximum number found in the material studied. The sporophyte is of the slender, regular type. FIGS. 19 and 20. Later stages of development. In the embryo shown in figure 20 the foot proper will develop above wall *x–x*. FIG. 21. Basal portion of young embryo at later stage than figure 20. The foot proper is beginning to develop along the region *b–b*. All × 180.

An interesting situation is illustrated in figures 24–26. When the dissections were first begun, a large percentage of the young sporophytes were found to be disintegrating. This disintegration, in all the cases observed, begins with the apical cell and works downward. The cause



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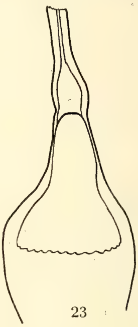
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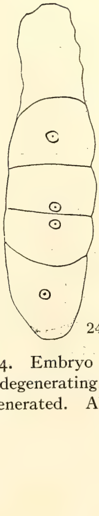
FIG. 22*a*. Young embryo beginning to digest the cells of the venter. FIG. 22*b*. The embryo has bored its way entirely out of the venter and lies embedded in the stalk of the archegonium. FIG. 23. Later stage showing the spreading of the foot. All × 60.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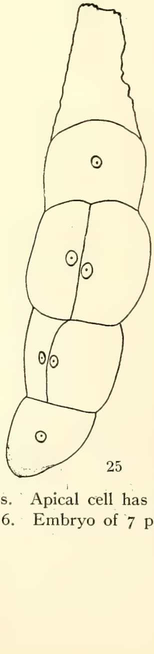
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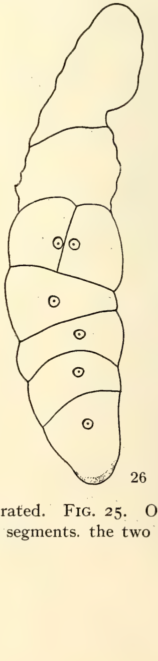
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FIG. 24. Embryo of 5 cells. Apical cell has degenerated. FIG. 25. Older stage, apical cell degenerating. FIG. 26. Embryo of 7 primary segments. the two uppermost having degenerated. All × 300.

could not be discovered. No fungus could be observed on or about the archegonia in these particular cases. The cells which had not disintegrated were apparently normal in every respect. It was unquestionably similar observations which led Schimper to state that it is the lower part of the young embryo which finally produces the sporophyte, the upper part dying and being resorbed.

Summary

The main points of this paper may be summarized as follows:

1. The fertilized egg divides by a horizontal wall into two approximately equal cells. A filament of cells—6 or 7 in number—is usually formed before longitudinal divisions occur.

2. In the material studied the wall which separates the cells at the two-celled stage cannot be traced with certainty in the older stages, hence no exact statement can be made as to the contribution of each of these two cells in the development of the sporophyte.

3. It is reasonably certain that apical growth occurs.

4. The basal portion of the young sporophyte may have walls appearing in a regular or in an irregular order. As a result of the former process there is developed a long, slender type of young sporophyte; as a result of the latter a shorter, bulbous type.

5. The number of primary segments, *i.e.*, segments formed by walls transverse to the axis of the archegonium, has not been found to exceed twelve.

6. A considerable number of very young sporophytes show basipetal disintegration.

Conclusions

Much has been made of the striking character common to the Anthocerotales and the Sphagnales as contrasted with the remaining members of the Bryophyta—namely, the origin of the sporogenous tissue from the endothecium. The writer desires to point out the wide difference in early embryogeny. The general history in the Anthocerotales is the formation of an unequal quadrant, the two upper cells being somewhat larger than the basal ones. This stands in sharp contrast to the filament of six or seven cells produced in Sphagnum. An examination of the early embryogeny of the Bryophyta shows that the closest approach in this respect to the condition in Sphagnum is to be found among the Jungermanniales. Here a filament of three cells formed before the appearance of vertical walls is not uncommon.

If similarity in early embryogeny is significant in determining phylogeny, it must be evident that in this one respect Sphagnum shows a closer relationship to the Jungermanniales than to any other group of the Bryophyta.

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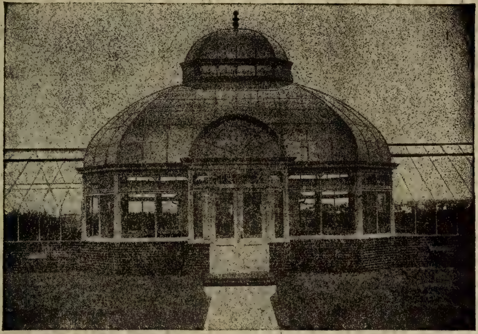

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Yours sincerely
Byron D. Halsted

AMERICAN
JOURNAL OF BOTANY

VOL. VII OCTOBER, 1920 NO. 8

BYRON DAVID HALSTED[1]

June 7, 1852–August 28, 1918

F. L. STEVENS, L. H. PAMMEL, AND MEL T. COOK

"I loved Byron for the genuineness of his religious faith, for the simplicity and beauty of his relationship to his fellows, for his ardent desire for service to the world and catholic tolerant spirit exercised toward those who differed from him in faith and thought." This appreciation of Dr. Halsted by an intimate friend will find response in the hearts of all who knew him. However brilliant the achievements of the individual from the world viewpoint, whether in finance, art, literature, or science, it is to the personality, the characteristics, traits, inclinations, and moralities, that we turn in making the ultimate estimate of the man. Dr. Halsted's genial nature, generosity, patriotism, and broad interest in art, music, literature, and athletics, as well as his scientific attainments, are the attributes that claim our homage.

He was one of the few of America's eminent pioneers in plant pathology, the first graduate student to take work under Dr. W. G. Farlow, the first to take the doctorate in cryptogamic botany at Harvard. He taught plant pathology at Ames when the subject was in its infancy in America, and there also he began a series of publications on plant pathology. Indefatigable and full of enthusiasm as a worker and keen as an investigator, a bibliography of his titles would number approximately four hundred, with contributions chiefly to plant pathology and plant breeding.

Dr. Halsted entered Michigan Agricultural College in 1867 and was graduated in 1871. He entered Harvard University in 1874 and received the degree of Doctor of Science in 1878 with a thesis on the "Classification and Description of the American Species of Characeae." He was managing editor of the *American Agriculturist* for five years, then went to the chair of botany of the Iowa Agricultural College, which position he held from 1885 to 1889, when he was elected to the professorship of botany at Rutgers College and the position of botanist of the New Jersey Agricultural College Experiment Station. In both institutions he endeared himself to students and faculty and laid broad, enduring foundations for botanical departments.

---

[1] Prepared at the request of the council of the Botanical Society of America.

[The **Journal** for July (7: 261–304) was issued August 6, 1920.]

Many students owe their later successes in life and their contributions to the world's welfare to inspiration and ambition derived through contact with Dr. Halsted in these two colleges.

His interest centered primarily in plant pathology, and pioneer work was carried on regarding many fruit and truck crop diseases. He also gave special attention to the diseases of ornamental plants, a field in which his work probably overshadows that of all other contributors. While in New Jersey failing eyesight and health forbade further microscopic work and his activities were turned to field work in plant breeding.

Dr. Halsted was a member of Phi Beta Kappa. He also won the silver medal of the Massachusetts Horticultural Society and was a fellow of the American Association for the Advancement of Science. He served in an official capacity many of the leading national scientific societies of the country. He was elected president of the New Jersey Microscopical Society, second vice-president of the Iowa Academy of Science in 1888–1889, secretary of Section F of the American Association for the Advancement of Science in 1892, secretary of the Society for the Promotion of Agricultural Science in 1892, and its president from 1897 to 1899, and was president of the Botanical Society of America in 1900–1901. He was affiliated with other national societies—the Society for Plant Morphology and Physiology, the Society of Horticultural Science, the American Society of Naturalists, and was associate editor of the *Bulletin of the Torrey Botanical Club* (1890–1893) and a contributor to the *Systematic Flora of North America.*

Dr. Halsted was born at Venice, New York, of Quaker parentage June 7, 1852; was thrice married and leaves three children. He died at New Brunswick, New Jersey, the scene of his activities of nearly thirty years, on August 28, 1918.

PUBLICATIONS

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1877

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1878

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1881

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Barn plans and outbuildings. 235 pp. New York.

1883

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Notes on sassafras-leaves. Science **2**: 491–493.

A strange sassafras-leaf. Science **2**: 684, 685.

A combination walnut. Science **2**: 761, 762.

1884

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Retrograde metamorphosis of a strawberry-flower. Science **3**: 302.
Conditions of growth of the wheat-rust. Science **3**: 457, 458.
Mildew and grape rot. Proc. N. J. State Hort. Soc., 9th Ann. Meeting (1884): 74–76.

1885

A tomato disease. Proc. Soc. Prom. Agr. Sci., 5th Ann. Meeting (1884): 42–44.

1886

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Gymnosporangium macropus on *Pirus coronaria*. Bot. Gaz. **11**: 190, 191.
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A pleasing experiment in laboratory practice. Bot. Gaz. **11**: 339, 340.
Strange pollen-tubes of Lobelia. Amer. Nat. **20**: 644, 645.

1887

Pollen-tubes of Lobelia. Amer. Nat. **21**: 75, 76.
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1888

Bulletin from the Botanical Department of the State Agricultural College, Ames, Iowa. 118 pp.
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Iowa Peronosporeae and a dry season. Bot. Gaz. **13**: 52–59.
Pollen germination and pollen measurement (Abstr.). Bot. Gaz. **13**: 238.
Trigger-hairs of the thistle flower. Bull. Torrey Club **15**: 82–84.
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Apple rusts. Rept. U. S. Comm. Agr. 1888: 370–381.
Potato flowers and fruit. Proc. Soc. Prom. Agr. Sci., 9th Ann. Meeting (1888): 33, 34.
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1889

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Another Sphaerotheca upon Phytoptus distortions. Journ. Mycol. **5**: 134.
Some notes upon economic Peronosporeae for 1889 in New Jersey. Journ. Mycol. **5**: 201–203.
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Peronospora upon cucumbers. Bot. Gaz. **14**: 152, 153.
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Notes upon Lithospermum. Bot. Gaz. **14**: 202, 203.
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Reserve food substances in twigs (Abstr.). Bot. Gaz. **14**: 260.
The station botanists at Washington. Bot. Gaz. **14**: 305–309.
The germination of pollen. Bull. Torrey Club **16**: 130, 131.
Observations upon pollen measurements. Bull. Torrey Club **16**: 135, 136.
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Some fungus diseases of the cranberry. N. J. Agr. Coll. Exp. Sta. Bull. 64. 40 pp.
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Our worst weeds: A scale of points. Proc. Soc. Prom. Agr. Sci., 10th Ann. Meeting (1889): 43–49.
The potato rot. N. J. Agr. Coll. Exp. Sta. Spec. Bull. G. 4 pp.
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The sweet potato rot. N. J. Agr. Coll. Exp. Sta. Spec. Bull. J. 3 pp.

1890

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Peronospora rubi Rabenh. in America. Bot. Gaz. **15**: 179.
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Hollyhock diseases. Garden and Forest **3**: 158.
Why not legislate against the black knot? Garden and Forest **3**: 194.
Anthracnose or blight of the oak. Garden and Forest **3**: 295, 296.
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1891

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The hydrangea blight. Garden and Forest **4**: 177.
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An orchid anthracnose. Garden and Forest **4**: 309.
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Gall-worms injuring the roses. Garden and Forest **4**: 608.
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What the station botanists are doing. Bot. Gaz. **16**: 288–291.
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1892

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"Falling" of egg-plant seedlings. Garden and Forest **5**: 164.
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Check list of American weeds, in the order of Patterson's list, exclusive of sub-tropical Florida. New Brunswick, N. J.
Fungi injurious to weed seedlings. Proc. Soc. Prom. Agr. Sci., 13th Ann. Meeting (1892): 145–148.
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1893

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Some new weed fungi. Bull. Torrey Club **20**: 250–252.
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Fatal club-root of turnips. Garden and Forest **6**: 78, 79.
A serious filbert disease. Garden and Forest **6**: 134.
An observation on fruit decays. Garden and Forest **6**: 342.
The rust of mountain ash. Garden and Forest **6**: 508.
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1894

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1895

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1896

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The black knot of the wild cherry. Forester **2**: 39, 40.
Notes on agriculture and horticulture (IV.). Science, n. ser. **3**: 398, 399.
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1897

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1898

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Rusts, smuts, ergots and rots. Some of the diseases that seriously affect field crops, vegetables and fruit. Remedies that have proved successful.

ABSORPTION OF MOISTURE BY GELATIN IN A SATURATED ATMOSPHERE[1]

Charles A. Shull and S. P. Shull

A great deal of work has been done to determine the relation of colloidal matter to water, gelatin being a favorite substance for such studies. However, one finds but few attempts to determine the water relations established when the colloidal material is exposed to an atmosphere saturated with water vapor. The earliest observations of a difference in behavior of colloids toward water and water vapor are credited to Volbehr (5). The main work dealing with this subject is a paper by von Schröder (3), who claims that gelatin absorbs much more water when placed in liquid water than when exposed to a vapor-saturated atmosphere. The data presented in his paper were afterwards used as a basis for a theoretical discussion by Bancroft (1), who attempted an explanation of the observed phenomena, but without any apparent attempt to verify von Schröder's results. One finds in the recent literature occasional reference to these papers, as in Czapek (2). Here Czapek (p. 42) adopts Bancroft's explanation of the supposed difference between the vapor pressure of the colloid and that of the mass of water which saturates the atmosphere about the colloid.

For the sake of clearness it will be advantageous to state briefly the results of von Schröder's investigations as given in section VII of his paper, which is entitled "Ein Beitrag zur Thermodynamik der Quellung" (*l.c.*, pp. 109–117).

In the first place, gelatin absorbs water very rapidly from liquid water. A piece of gelatin weighing 0.801 g., and which contained 17.6 percent of hygroscopic water, took up moisture as shown in table 1.

Table 1. *Absorption of Water by Gelatin from Liquid Water*

Time	Intake in Grams	Gain Percent*
5 mins.	2.282	336.1
10 mins.	2.934	432.1
20 mins.	3.669	540.3
30 mins.	4.072	599.7
40 mins.	4.300	633.3
50 mins.	4.415	650.2
60 mins.	4.506	663.6
2 hrs.	4.941	727.7
24 hrs.	6.911	1018.
48 hrs.	7.734	1039.

* Calculated on absolute dry weight of gelatin disc, 0.679 g.

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[1] Contributions from the Botanical Laboratories of the University of Kentucky, No. 3.

But if a similar piece of gelatin is placed in a saturated atmosphere, moisture equilibrium between gelatin and water is reached at a much lower percentage of intake, as shown in table 2. In this case the air-dry gelatin disc weighed 0.904 g.

TABLE 2. *Absorption of Water by Gelatin in a Saturated Atmosphere*

Time	Intake in Grams	Gain Percent
1 day	0.154 g.	17.08
2 days	0.218	24.10
3 days	0.277	30.69
4 days	0.294	32.56
5 days	0.347	36.21
7 days	0.357	39.52
8 days	0.366	40.52
15 days	0.372	41.18
17 days	0.368	40.74
18 days	0.369	40.80
20 days	0.374	41.41

These figures make it appear that equilibrium was reached at about the end of a week, at a little over 40 percent of absorption.

When the gelatin was first soaked in water till nearly saturated, and then brought into a saturated atmosphere, there was continuous loss of water from the gelatin. Thus a piece of gelatin weighing 0.433 g. was soaked until it weighed 5.092 g. It was then placed in a chamber with a supposedly saturated atmosphere. The behavior is shown in table 3. The first three columns to the left, except the top line, are taken from von Schröder, and the two columns to the right are from Bancroft's discussion of the same experiment, with a correction made by omitting the dashes in Bancroft's table, and lifting the figures into correct alignment with the time intervals.

TABLE 3. *Loss of Water by Gelatin in a Saturated Atmosphere*

Time	Loss in Weight in G.	Loss Percent	Weight of Water in Gelatin in G.	Percentage Absorbed Water Remaining
0 day	——	——	4.659	1,076
1 day	0.259	6.29	4.400	1,016
2 days	0.337	7.82	4.332	998
3 days	0.383	8.87	4.276	988
4 days	0.418	9.68	4.241	979
5 days	0.929	21.50	3.730	861
7 days	1.313	30.45	3.346	759
9 days	1.972	45.63	2.687	621
11 days	2.571	59.48	2.088	482
14 days	3.175	73.46	1.484	343

The percentages in column three are not calculated with great accuracy, but are presented exactly as in the original. The data indicate that there is continuous loss of water from the gelatin, although the air on all sides is assumed to be saturated. The behavior implies a contradiction to the second law of thermodynamics. Such an evaporation of water from one part of the system to another under the conditions could take place only if the colloidal matter had a higher vapor pressure than the liquid water; and as the water evaporated from the colloidal mass, it would have to condense at the surface of the liquid water. Bancroft's explanation of the higher vapor pressure is based upon the shape and size of the water droplets in the gelatin. They are assumed to be round, and it is held that water evaporates more readily from a curved surface than from a flat one. The droplets of water are so minute that the curvature of the surface of each droplet is quite sharp; this results in a vapor pressure higher in the colloid, and a consequent distillation of water from it to the liquid phase of the system. The distillation should go on until in some way or other the vapor pressure throughout the system reaches equilibrium.

Being engaged in the study of the relation of certain colloidal organic substances to water, we have had occasion to perform some experiments which led to a repetition of some of von Schröder's work. However, it was not possible to tell from von Schröder's discussion just how he set up his experiments, how he controlled the temperature, and how he secured and maintained saturation. In a number of ways the discussion leaves one in the dark, and we were compelled therefore to work out a method of investigation which no doubt differs in a number of ways from von Schröder's. The methods employed are briefly stated.

Materials and Methods

The gelatin used in the experiments to be recorded here was the Gold Label gelatin commonly handled by dealers. No attempts were made to purify it in any way; for, although pure substances are to be preferred in original work of any kind, we felt justified in using the gelatin in its commercial condition because the experiments we were trying to repeat had been carried on with unpurified gelatin. There is no doubt that von Schröder's gelatin as well as ours contained acids, and that in both cases salts were present. We could have neutralized our gelatin with some inorganic base, with subsequent dialysis until it was salt-free. This would have given us a purer gelatin, but we could not then have made a direct comparison between our own and von Schröder's results. It seemed to us best therefore to repeat the experiments with ordinary gelatin, as the differences between the two substances would likely be less than if we used any purification method to remove acids and salts.

During the earlier tests the gelatin was prepared by dissolving it at gentle heat in distilled water, after which it was poured into petri dishes which had been slightly smeared with glycerol to prevent the gelatin from sticking to the glass; but as the glycerol is hygroscopic, this method was abandoned to avoid contamination of the gelatin with glycerol. Mercury was used as an agent to prevent the gelatin from sticking to the glass during drying. In this way sheets of gelatin of desired thickness could be secured, and these sheets were cut into disks before they became entirely dry, while still slightly pliable, and the disks were dried out in contact with air until weight loss ceased. During this preparation the gelatin was carefully protected from dust to reduce chances of mold or bacterial infection.

The disks were exposed to a saturated atmosphere in small wide-mouthed bottles. The bottles were arranged with a layer of mercury in the bottom sufficient to sink them. Over the mercury was a layer of water. The gelatin disks were suspended over the water, just as near the water surface as practicable, in shallow paper baskets which were attached by threads and wax to the center of the rubber cork which closed the bottle. The rubber cork was shellacked upon its inner and outer surfaces to protect it from water, probably an unnecessary precaution.

To control the temperature the bottles were sunk in a Freas thermostat which was set to run at 26° C., and which showed no deviation with an ordinary chemical thermometer during the five months while the tests were run. It was found necessary to control the growth of bacteria and molds on the gelatin, and this was accomplished by the use of small pieces of thymol in the water. The bottles were usually placed in the thermostat for some time before the gelatin was introduced. This allowed the air to become more nearly saturated. Then the weighed disks were put into the baskets. At intervals which were purposely made infrequent so as not to interfere with the saturation of the air, the disks were removed carefully and quickly to weighing bottles and weighed. The greatest care was taken to keep the disks from drying out during weighing, and to keep the air to which they were exposed during intake intervals from becoming unsaturated. The bottles were always corked and returned to the thermostat during weighings. It was noted that there was always condensation of vapor on the walls of the weighing bottles, so that it was not possible to prevent all losses of water.

Results

In all cases it was found that much more water was absorbed by the gelatin from the atmosphere than von Schröder had observed. During the preliminary tests in one instance there was a regular intake of water which continued for weeks, and which had reached an intake of 250 percent of the weight of gelatin when it was accidentally overturned by the laboratory attendant, and the experiment was thus brought to an abrupt end. A considerable number of disks were started, some with and some without thymol. The rate of intake was apparently about the same during the first several days, but the disks exposed without thymol would always suffer in time with bacterial or mold infections. Usually after about three days the uncontrolled disks had to be discarded. The indications were that the thymol itself was not noticeably accelerating the rate of water intake. Plotted curves practically coincided during the first several days, with and without control.

One typical example of the intake has been chosen to illustrate the behavior of the gelatin under the given conditions. The gelatin disks weighed 0.787 g. air-dry. The absorption data obtained during 47 days are given in table 4.

TABLE 4. *Absorption of Water by Gelatin in a Saturated Atmosphere.*

Time	Intake in Water	Gain Percent
6.5 hrs.	0.1292 g.	15.27
16.5 hrs.	0.2174	27.62
1.0 day	0.2698	34.28
2.0 days	0.3796	48.23
3.0 days	0.4599	58.43
4.0 days	0.5332	67.75
5.04 days	0.5884	74.76
6.0 days	0.6260	79.54
8.0 days	0.7109	90.20
10.0 days	0.7754	98.52
12.0 days	0.8356	106.17
14.0 days	0.8734	110.97
18.0 days	0.9644	122.54
22.0 days	1.0374	131.81
26.0 days	1.0979	139.50
30.0 days	1.1602	147.42
35.0 days	1.2209	155.13
40.0 days	1.2886	163.73
47.0 days	1.3426	170.59

After the last weighing recorded in table 4 was made, the disks were returned to the bottles and left undisturbed for two months, as they were hard to handle without breaking. On opening them at the close of two months, the disks were found in liquefied condition. No culturing was attempted to determine whether liquefying bacteria might have been present. But the thymol had inhibited development of molds, and it is usually considered that molds are less readily controlled than bacteria. It does not appear to us likely, therefore, that liquefaction was brought about by bacterial action.

The contrast in behavior of gelatin as we have found it, and that reported by von Schröder, is shown graphically in figure 1. The possible cause of this difference will be considered later.

One striking thing which has been noted in regard to water absorption is the regularity of intake. It usually begins rapidly, and becomes continually less rapid as absorption increases, falling off in regular fashion till approaching saturation causes a more rapid decline in the rate of intake. The rate of absorption has been studied particularly with reference to seed colloids soaking in water, and mathematical analysis has given us a formula by which the intake can be closely approximated by calculation.

The same kind of analysis was made of the gelatin absorption from saturated air, and the same formula that was derived from the absorption of water by seeds from liquid water can be used in calculating the curve of absorption of water by gelatin from a saturated atmosphere.

The generalized formula is $y = a \log_{10} (bx + 1) + c$, in which y is the percentage of total intake, and x the time, with a, b, and c constants.

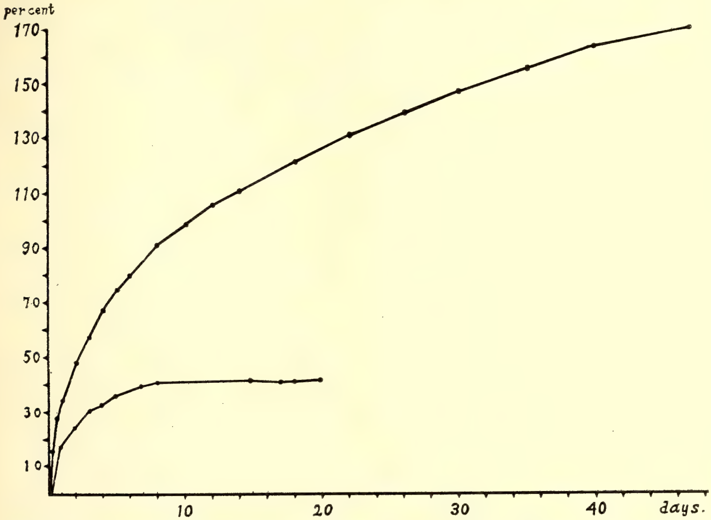

FIG. 1. Curve of moisture intake of gelatin in a saturated atmosphere. Lower curve, from von Schröder's data. Upper curve, from data presented in Table 4.

In the case of seeds we found it necessary to use two or even three curves to approximate the experimental data (4). Similarly it is necessary to use two equations for curves joining in a common tangent value to express the gelatin absorption curve.

The first part of the curve, with the values of a, b, and c substituted in the equation, is as follows: $y = 93.4 \log_{10} (0.032x + 1) + 10.413$; while the later part is expressed thus: $y = 141.9 \log_{10} (0.0064x + 1) + 40.82$. These two curves have equal tangents at $x \doteq 209.47$ hrs., at which time the two values for y are, $y_1 = 93.22723$, and $y_2 = 93.22764$, showing a break in the curve of only .00041 per cent. In other words, at the end of about six days, when the gelatin has taken in something less than its own weight of water (93 percent) it requires different values for the constants in the general formula to keep a calculated curve running close to the data. With these two successive curves there is fairly close agreement between the calculated and the observed data as shown in table 5.

The agreement is very good with the exception of the first and the next to the last readings. The data at 960 hours may be in error, although there is nothing in the records to indicate it. It seems scarcely likely that the data which had been running so regularly during the preceding 500 hours would suddenly rise above the curve, and then drop back again to the curve 168 hours later. If the error involved accidental addition of the water to the gelatin, it should show in the last reading also. If an error was made it was most likely an error in counting the weights, as a reduction of 10 milligrams in the weight would bring the data to 162.46, which would bring fair agreement with the calculated value. But even as the figures stand the agreement is very striking, and shows that water intake goes steadily forward at a rate determined by the conditions of the experiment.

TABLE 5. *Agreement of Calculated and Observed Intake by Gelatin from Saturated Atmosphere*

Time	Data Low	Calculated Intake	Data High
6.5 hrs.	15.27	18.08	
16.5 hrs.		27.61	27.62
24 hrs.		33.53	34.28
48 hrs.		48.16	48.23
72 hrs.	58.43	58.89	
96 hrs.		67.37	67.75
121 hrs.		74.64	74.76
144 hrs.	79.54	80.35	
192 hrs.		90.17	90.20
		Break in curve	
240 hrs.		98.17	98.52
288 hrs.		105.22	106.17
336 hrs.	110.97	111.54	
432 hrs.		122.52	122.54
528 hrs.	131.81	131.82	
624 hrs.	139.50	139.93	
720 hrs.		147.08	147.42
840 hrs.		154.98	155.13
960 hrs.		161.99	163.73
1,128 hrs.	170.59	170.62	

The actual rate of intake has been measured at several points by measuring the tangents to the calculated curve, and we give the rate of intake in grams per minute.

When y = 25 percent, velocity of intake is .015101.
When y = 30 percent, velocity of intake is .013348.
When y = 35 percent, velocity of intake is .011672.
At the break, y = 93 percent, velocity of intake is .002808.

The rate at the time the break occurred was approximately one fifth of the rate when 25 percent had been absorbed. The steady fall in the rate of intake should be noted. These rates are very much lower, of course, than when gelatin is immersed in water, and must depend partly at least on the surface-volume relation of the particular pieces of gelatin used. One would expect a thin, flat piece of gelatin to absorb more rapidly than an equal mass in spherical form.

An important difference between the curve of absorption as shown for gelatin in saturated vapor and that obtained for seeds in water should be noted. The absorption curve for Xanthium seeds shows a break at about 35 percent due to approaching saturation. The gelatin curve here presented shows no such break due to approaching saturation, but maintains a slowly decreasing rate over long periods of time, with remarkable regularity.

Discussion

The data which are presented indicate that gelatin absorbs much more water from a saturated atmosphere than was found by von Schröder. And an examination of his data leads one to suspect that he did not maintain a saturated atmosphere. It is not so easy to see in table 2, but in table 3, which records the loss of water from saturated gelatin, it is rather easily detected. If the behavior in this case were normal, we should find the loss largest during the first 24 hours, because the difference in moisture equilibrium was greatest at the beginning of the experiment. Each day thereafter should show less and less daily loss because of the closer and closer approach of equilibrium conditions. This, however, is not the case in his data. On the first day the loss is 0.259 g., on the second day 0.078 g., on the third day 0.046 g., on the fourth day 0.035 g., and on the fifth day 0.511 g. Counting average daily loss, there was nearly twice as large average daily loss at the end of five days as at the end of the first four days. And at the end of 14 days the average daily loss was still more than double that at the end of the first four days. From the fifth day on, the loss is always much more rapid than during the second, third, and fourth days. This would make it appear very probable that the gelatin was losing water into an unsaturated atmosphere.

And if the atmosphere is unsaturated in this case, it probably was unsaturated in the experiments, the data of which are recorded in table 2. It is a very difficult matter to produce and maintain conditions of saturation, and there is not much doubt that the frequent opening of the chamber for weighing allowed the atmosphere to fall considerably below the saturation point. There is nothing in von Schröder's discussion to show how he handled his materials. Even in our own work we can not be certain that complete saturation was procured and maintained at all times. But the results would indicate that we came nearer to it than did von Schröder.

We feel that the amount of work done is insufficient to show that colloids do not exhibit the phenomenon to which von Schröder's work called attention. Even though we have shown that gelatin takes up from saturated vapor much more water than was formerly supposed, it may still be true that colloids show a difference in behavior toward water in liquid and in gaseous form. Much of the difference in the actual amount of water taken in must be related to the filling of minute lacunae when immersed in water, and the saturation of the gelatin around the lacunae only, when exposed to vapor. The vapor pressure of a saturated colloid may actually be greater than that of a flat surface of water, and the colloid be able therefore to distil water into the saturated atmosphere which is common to both. Whatever the truth may be in regard to this point, it seems to us unfortunate that a theory of physical chemistry should be based upon a single piece of work which shows somewhat gross irregularities in the data, without any attempt to confirm the original findings.

The experience we have had with gelatin in saturated atmosphere, with conditions controlled as carefully as possible, at least suggests the desirability of a reinvestigation of the relation of the vapor pressure of colloids to that of the vapor pressure of water, before we try to establish or accept theories which attempt to explain this relation. It might be found that there is little to explain.

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SLOW AND RAPID GROWTH[1]

H. S. Reed

The growth rate of plants and of plant organs resembles the rate of a monomolecular chemical reaction (Reed, 1920 *a*). Having obtained a mathematical expression of the growth rate, it should be possible to analyze the process into some of its main components. It has been found that the growth rate of certain organisms may be expressed by the differential equation

$$\frac{dx}{dt} = k(a - x),$$

where x represents the size of the organism at time t, a represents the final size attained, and k is a constant of the reaction. The rate at any given time is, therefore, proportional to the amount of growth yet to be made. It is accordingly rapid at the outset and becomes slower as the end of the growth period is reached.

The integral form of this equation is

$$x = a(1 - e^{-kt}),$$

from which the size of the organism at any time may be calculated. If the above assumption is correct, the calculated value of x should not be widely divergent from the observed value for the same time. As a matter of fact, the two values have been found to agree very well. It seems profitable to extend this method of inquiry into different phases of the problem of growth, in the attempt to gain further information on the dynamics of growth.

Measurements of a selected number of shoots on young apricot trees were made throughout the growing season. The mean length of the shoots at each interval of measurement was taken as the observed length at that particular time.

The shoots were of two sorts, and measurements were separately made upon each. The first were on trees which received no pruning; the second were on trees which received, annually, a severe pruning, with the result that the new shoots grew very much more rapidly than those on the unpruned trees. Both classes of trees are in adjoining rows in the orchard and receive the same cultural treatments with the exception of pruning.

At the outset, 50 shoots were selected for each class, but the number was reduced by various accidents during the summer, with the result that there were 33 shoots in the unpruned class and 28 in the pruned class. In either case the number of variants is large enough to give a satisfactory mean.

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[1] Paper 70, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

TABLE I. *Mean Length of New Shoots on Pruned and Unpruned Apricot Trees During one Season*

t	On Unpruned Trees		On Pruned Trees	
	x (Observed)	x (Calculated)	x (Observed)	x (Calculated)
weeks	cm.	cm.	cm.	cm.
1	9	10	13	23
2	17	20	37	43
3	25	28	60	61
4	29	36	73	78
5	34	42	88	92
6	42	48	102	105
7	50	54	113	117
8	57	59	121	128
9	63	63	132	137
10	68	67	142	145
11	71	70	148	153
12	77	73	156	160
13	79	76	163	166
14	82	79	174	171
15	83	81	177	176
16	84	83	182	181
17	85	85	186	184
18	86	86	190	188
19	87	88	194	191
20	88	89	197	194
21	89	90	200	196
22	90	91	203	199
24				
25	94	94	208	204
26				
27			210	207

Table I contains the observed lengths of the two classes of shoots on the successive weeks of measurement. The mean final length of shoots on the unpruned trees was 94 cm., and that of shoots on the heavily pruned trees was 210 cm. We may let 100 represent the limiting value of x_1 and 218 that of x_2. By a series of approximations the equation

$$x_1 = 100(1 - e^{-.11\,t})$$

was found to be satisfactory for the values of the shoots on the unpruned trees, and

$$x_2 = 218(1 - e^{-.11\,t})$$

for the shoots on the pruned trees. A graphic comparison of these values is given in figure 1.

It will be seen that the only difference between the two integral equations is in the value of the constant a. The value of k, the constant of the reaction, is the same in both cases. The values of x calculated from these equations (table 1) are seen to be very close to the observed values with few exceptions; the values, therefore, may be assumed to be approximately correct.

A series of values more nearly corresponding to the observed lengths may be obtained by the means employed in another study (Reed, 1920 *b*) of this kind, but the simpler equation gives satisfactorily close values and its use will contribute to clarity of discussion.

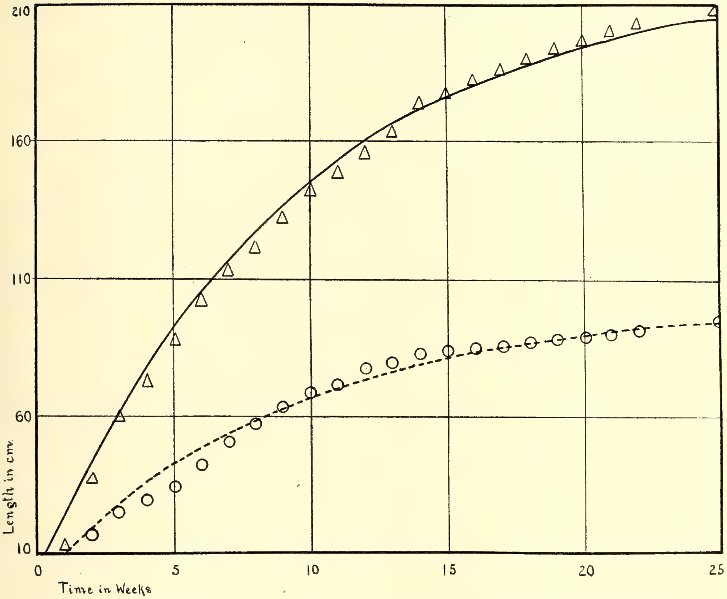

FIG. 1. Curves showing mean length of apricot shoots during one season.
△ △ △, Observed lengths of shoots on pruned trees.
————, Length of shoots calculated from $x_2 = 218(1 - e^{-.11t})$.
○○○○, Observed lengths of shoots on unpruned trees.
– – – – –, Length of shoots calculated from $x_1 = 100(1 - e^{-.11t})$.

The differential equation, $dx/dt = k(a - x)$, represents rate of growth, *i.e.*, amount of elongation in unit time. If we get the weekly increments in length, we shall have the observed increments in unit time expressed as a rate per week, and can compare them with values calculated from the above differential equation. Since there are inevitable fluctuations in the actual growth rate, it will be better to use "adjusted" values, S, of the observed increments. This is a *slope* method of determining the observed values of

dx/dt and has been applied to statistical problems by McEwen and Michael (1919). Its usefulness depends upon the fact that the slope of the chord of a simple curve is approximately equal to that of the tangent at the point midway between the extremities of the chord. The values of S are obtained from $\frac{1}{2}$ (observed length at time $t + 1$ − observed length at time $t - 1$), which represents the average rate between time $t + \frac{1}{2}$ and time $t - \frac{1}{2}$. Figure 2 shows the values so obtained compared with the calculated rate. They show that the rate is at a maximum at the inception of the growth period and follows the course of a curve decreasing exponentially to the end of the period.

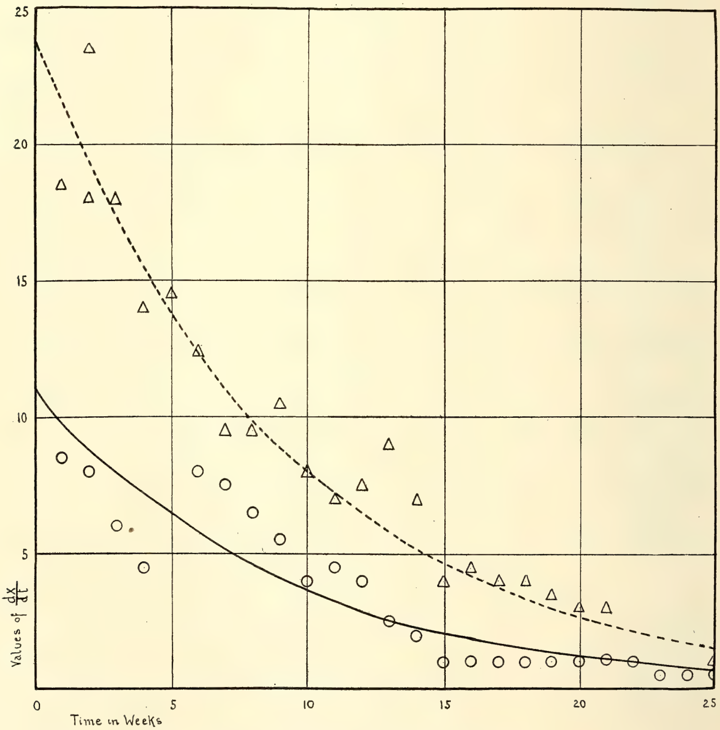

FIG. 2. Curves expressing rate of growth of apricot shoots.
△ △ △, Observed growth increments (S) of shoots on pruned trees.
– – – – –, Values of $dx/dt = .11(218 - x_2)$.
○ ○ ○ ○, Observed growth increments (S) of shoots on unpruned trees.
————, Values of $dx/dt = .11(100 - x_1)$.

From these results, it is plain that the quantitative difference between the two classes of shoots existed from the very outset, and that the greater total growth of shoots on the pruned trees was due to their faster growth in the early part of the period. This conclusion accords with the results of Pearl and Surface (1915), who showed that the superior plants in a population are, as a rule, superior from the seedling stage, and that the inferior members of the population are likewise inferior from the beginning.

This raises an important physiological question, *viz.*, How did the pruning of one lot affect the growth process in such a way that they made so much more rapid growth as soon as activity began in the spring? In other words, what happened to cause one lot to grow three times as fast as the other in the second week?

Referring to the differential equation expressing the rate, it will be seen that the rate in unit time is equal to the product of two quantities. The first quantity is k, the constant of the reaction, and the other is $(a - x)$, the difference between a constant and the length of the shoots at time t. The rate of growth of the two classes of shoots differs, then, only by the value of the second factor, *i.e.*, $(a - x)$. From the data, it seems probable that k, the constant of the reaction, is determined by the genetic constitution of the tree. It is well known that its value is determined from

$$k = \frac{1}{t} \log \frac{a}{a - x}.$$

The quantity $a - x$ is, therefore, the one whose value was altered. Now, from the integral equation

$$x = a(1 - e^{-kt})$$

it is easy to see that

$$a - x = ae^{-kt},$$

which means that the values of $a - x$ are equal to the product of a by an exponential function of the time. Since in both the unpruned and the pruned trees the value of e^{-kt} was the same, it is, therefore, plain that the value of $a - x$ is dependent upon the value of a. While the value of a must be, in a measure, determined by hereditary factors, it seems also subject to the influence of outer environmental factors such as those here operative.

In short, the rate of growth of the shoot appears to depend upon its final length. Whatever, therefore contributes to the production of the ultimate length of the shoot influences the rate of growth from the beginning of the season.

The close correspondence between the growth of the shoots and the equations above stated is evidence that their growth is some sort of a catalytic process. According to this view, the organism is the end-product of a process in which a catalyst acts upon a substrate.

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CYTOLOGY AND SYSTEMATIC POSITION OF PORPHYRIDIUM CRUENTUM NAEGELI

Ivey F. Lewis and Conway Zirkle

Porphyridium cruentum, named by Nägeli in 1849, has had a systematic history equaled by few plants. It had previously been called at various times Thelepora, Tremella, Sarcoderma, and Byssus. Agardh (cit. Brand) named it *Palmella cruenta*, and under this name Hassall classified it with the Palmellaceae. Indeed, Nägeli himself placed it in this group, and there it was kept by Kützing in his Tabulae Phycologicae (1849–71) under the name given it by Agardh. Rabenhorst seems to have been the first to place it in the Porphyraceae (1868), and he was followed in this four years later by Wood. Cooke in 1884 returned it to the Palmellaceae. Wolle likewise placed Porphyridium in the Chlorophyceae and found it to be identical with *Protococcus miniatus*. On the other hand, Schmitz (cit. Brand) considered it related to the Florideae, and Gaidukov (cit. Engler and Prantl) put it in the Bangiaceae. In 1902 Chodat returned it to the Chlorophyceae and would place it near Schizogonium. West two years later believed that Porphyridium belongs in the Myxophyceae and is allied to Aphanocapsa, while Hansgirg (cit. DeToni) made the genus but a species of Aphanocapsa and called it *A. cruenta*. Oltmanns in 1905 put Porphyridium once more in the Chlorophyceae. He was not certain as to its exact position but placed it supplementary to the Scenedesmaceae. DeToni classified Porphyridium as one of the Myxophyceae belonging to the family Glaucophyceae. Brand in 1908, as a result of his work on this plant, believed that it belongs to the Bangiaceae, and in this he is generally followed by the systematists, Engler and Prantl, West, who changes his original position, and Collins. Tilden, however, keeps Porphyridium in the Myxophyceae. While Kufferath got some results very different from Brand's, he agreed with the latter as to its systematic position, although he suggested that in the contingency of its having no chlorophyll it be placed with the red bacteria. Brand cited Borzi as being in favor of putting Porphyridium with Protococcus, Richter as favoring putting it with Trentepohlia, while Klebs would have it as a questionable member of the Pleurococcaceae.

The descriptions of Porphyridium differ almost as much as its various systematic positions. Nägeli, working unfortunately with dried material, described it as follows:

"Cells flattened, in surface view round or somewhat polygonal from lateral pressure, with a lateral thin confluent sheath, united in one-layered free-lying families; divisions in varying vertical planes; all generations fully developed and alike; cell contents purple.

"Type *P. cruentum* (*Palmella cruenta* Ag.), the only known species.

"The blood-red gelatinous layer consists of larger or smaller one-layered plates, whose cells seen from the surface appear rounded and mostly somewhat angular. The thickness of the cells is in dry specimens one third to one fifth the breadth. The thin sheaths run together in a structureless jelly in which the cells are imbedded. The sheaths are one third to one fifth, more seldom up to one half, of the lumen. The true wall is very thin.

"The cell content is colored by erythrophyll. It looks beautifully purple and agrees in color with *Porphyra vulgaris*. I could not see a nucleus in it."

On procuring some living material he amended his description somewhat:

"Cells spherical or polyhedric with tolerably thin confluent sheaths, united in a somewhat gelatinous layer; divisions varying in all directions of space or exceptionally only in vertical planes. . . . This genus is distinguished from Palmella by the erythrophyll in the cell content." He added in a note: "Further I saw in the fresh plant, often in every cell, a whitish granule (a chromatophore filling itself with starch), such as the other Palmellaceae possess."

In 1875 Mer found starch in Porphyridium, and in the same year Saint-Léon found no trace of sexual reproduction and only simple multiplication through the division of the vegetative cells. Schnetzler (1878) reported that the red coloring matter disappears when the alga is pickled in a borax solution, leaving the color green, and Nebelung (1878) that the red pigment has a spectrum which may be considered as a modified spectrum of the pigment of Phormidium. Schmitz added considerably to our knowledge of this alga by describing in it a star-shaped chromatophore, which, like those of the Bangiaceae, Bacillariaceae, and Rhodophyceae, contains no starch; and also a colorless centrally located pyrenoid and an eccentric nucleus. Later he reported that "the special cell membrane is repeatedly formed anew on the single cells, the old membrane is torn through on one side and stripped off as a stalk, at first sharply delineated and later becoming more and more formless gelatin." On the other hand, Oltmanns described the cells as being imbedded in formless jelly.

Brand reported that the chromatophore is not typically star-shaped but often in wet weather is round, and that the star-shape, when it does occur, comes from its being indented with the peripherally located granules and vacuoles. These granules he took to be cyanophycin granules, though he records that they are not stainable with acid carmin, which is generally held to be the most typical stain for such granules. The coloring matter, he found, is floridean red and varies only in its intensity. He was unable to find any green modification. The pyrenoid is described as being ring-shaped and often hard to see, and in "house cultures" it even disappears in most cells. In regard to the nucleus he said: "Although now the existence of a nucleus is *a priori* very probable, I could, after completely dissolving the sheath, never with certainty show one. The nucleus-like structures which one sees in living as well as in fixed stained material, are not only in regard to size, form, and position very variable, but appear sometimes single and sometimes many. All usual methods of staining have given me, through repeated investigations, very uncertain results."

Kufferath, utilizing the technic of bacteriology, was able to get a pure culture of Porphyridium to grow in various gelatinous media. The alga growing thus showed a great increase in size, at times reaching a diameter of 24 μ, and showed somewhat of a variation in its method of division. Two daughter cells sometimes developed within the body of the mother cell, and even tetrads occurred. Kufferath denied the existence of a pyrenoid in Porphyridium and stated that what has been taken for a pyrenoid is an optical effect due to a convergence of the light rays by the plastid. In regard to the nucleus also his findings are quite different from Brand's. He writes: "The nucleus, which has been seen only by Schmitz, is colored by the usual stains; it is oval, somewhat refractive and applied against the cell wall; it is small and we have not been able to distinguish its intimate structure."

A most obvious explanation for this divergence in the results of the various investigators would be furnished if the case of Porphyridium were analogous to that of Protosiphon and Botrydium. Different species of plants, no matter how much alike externally, would hardly give identical results on an intimate investigation, especially if their ancestry were diverse and they had evolved along parallel lines. While it is possible that more than one genus has been investigated under the name of Porphyridium, and this possibility should not be overlooked in future investigation of this much studied but little known alga, the facts at present do not substantiate this hypothesis. The present investigation has often shown in the same plant two characters, each of which has been described and had its existence denied by some of the aforementioned authors, whose views were just the opposite in regard to its accompanying character.

The diameter of Porphyridium in the material studied varies from 5 μ to 9 μ, the smaller cells almost uniformly being in the resting condition. The jelly secreted by each cell forms an individual sheath about that cell and, when division takes place, the two daughter cells are in the same sheath, which follows the constriction of the cells quite intimately, and lengthens as the cells draw apart. The portion of the sheath between the two cells becomes drawn out into a strand or stalk (figs. 10, 11, 39). As these cells were originally within the sheath of the mother cell, which itself was on a stalk, we frequently find the mother stalk branching into two daughter stalks. However, no case was found of more than two cells being borne on branches on a single stalk, which would indicate that the stalk does not persist through three generations. Indeed, if growth is inhibited, the stalks tend to blend into a common gelatinous sheath and appear as in figure 12. The stalks are elastic. It is quite a common instance for two sister cells to have stalks of different lengths, and in each instance observed the longer stalk was the thinner, as if it had been stretched out. Brand observed that the pressure of the cover glass would flatten out the jelly, which would resume its original shape if the presure were removed.

Löffler's flagellum stain will show these stalks very well, a little better as a rule if pyrogallic acid be used in place of tannic acid. A good method of proceeding is to place a small amount of rapidly growing alga on a slide and allow it to dry until it has lost all of its water content. It should then be covered with the mordant and heated for ten minutes over a water bath.

Much clearer results, however, have been obtained by allowing the alga to dry as described above and then fixing in the following solution:

Sat. sol. anhydrous ferric bromide in ether	1 part
Molar sol. pyrogallic acid in ether	2 parts

The water in the gelatin will cause the solutes to ionize, and hence ink will be precipitated within the gelatin. This makes a good mordant for gentian violet and safranin. If the jelly has dried too much it can be impregnated with ink by having the fixing agent washed off with water. Another good fixing and staining agent for jelly is:

Sat. sol. gentian violet in 95% alcohol	1 part
Formalin (40% formaldehyde)	1 part

This stains the jelly a dark red or purple and leaves the cell contents colorless.

The chromatophore is typically star-shaped in the resting cell (figs. 12, 13, 18). However, in the cells that are rapidly growing, the enlargement of the cell does not seem to be followed by an equal increase in the size of the chromatophore, so large vacuoles appear at its periphery. Its shape can then be best described as amoeboid.

The chromatophore is of a dark red color, almost that of clotting blood. If, however, the plant is allowed to stand for a short while under water, the red coloring matter can be seen dissolved in the water and the gelatinous mass becomes grass-green.

The centrally located body, which has almost uniformly been called a pyrenoid whenever it was observed, and will be considered such in this paper, is colorless in the living cell and appears only as a light spot in the chromatophore. Unstained it could very readily be mistaken for an artefact due to the refraction of light by the chromatophore. However, the "convergence of light rays" of Kufferath takes Heidenhain's haematoxylin very well and is not indifferent to gentian violet and safranin (figs. 2, 9, 18, 27). The pyrenoid is generally spheroidal in shape, though when the cell starts to divide it lengthens and becomes somewhat angular. As a rule it stains uniformly dark, though at times it appears ring-shaped with a relatively unstained center (figs. 25–27).

A single eccentrically located globule, a trifle smaller than the pyrenoid, has been frequently noted in Porphyridium. It can be seen very easily in the living specimen and has been observed to fragment as the water content of the cell increases, the fragments arranging themselves about the chromatophore. Except in its reaction toward acid carmin it seems to act as if it were cyanophycin. In general, we find, it takes the usual nuclear stains, haematoxylin, gentian violet, and safranin.

Picric acid has, on the whole, given the best results as a fixing fluid. If it is washed out in running water, the chromatophore will be dissolved and the pyrenoid and "nucleus" left without being obscured by any other cell structure. Ten minutes in the acid is enough for the fixation, and from fifteen minutes to twelve hours will do for the washing out of the fixative. Mordanting for one hour in iron alum and staining for a like period in haematoxylin have given the best results with this stain. Staining for ten minutes over a water bath is sufficient for anilin-gentian violet and anilin-safranin, and the specimen can then be decolorized by allowing it to stand over night in methyl alcohol. Another very successful fixative and mordant is:

Pyrogallic acid (25% aqueous sol.)	10 parts
Ferrous sulphate (sat. sol.)	5 parts
Fuchsin (sat .alc. sol.)	1 part

Van Ermengen's osmic acid process has given only fair results. Whenever the material was fixed in either of Flemming's fluids, or when fixed in picric acid and hardened in alcohol, the chromatophore stained so densely that it was impossible to distinguish anything in the cells clearly. Flemming's triple stain has given very fair results.

The chromatin, consisting of a single eccentric granule surrounded by a clear space in the cell (fig. 18), is typical of the resting stage, a stage described by Brand as "wasserarm." The cell, however, if dried, is useless as far as any clear results are concerned. As the cell prepares for division, this granule enlarges and begins to fragment, assuming the various shapes shown in figures 18–27. No hard and fast rule can be laid down for establishing a sequence of forms in this breaking up, as there are many forms which do not fit well into any series that could be arranged out of the others, although some of the shapes occur in many cells. The "U" shape is perhaps the most common (figs. 21, 22), and it is not at all unusual to find the fragments united in a line (figs. 23, 24) or in a ring (fig. 20). Frequently the pieces in drawing apart leave trails which have a striking resemblance to the mitotic spindle (fig. 25), which resemblance seems to be purely accidental. As an end result of this fragmentation the chromatin is distributed in the form of small elongated granules about the periphery of the chromatophore (figs. 27, 28). These granules then fuse end to end and a well tangled spireme results (fig. 29). A striking thing about this spireme is the way various strands lie parallel to each other. One is greatly tempted to see in this a conjugation or perhaps a splitting of the spireme. However, the members of a pair do not necessarily go to different cells. The spireme breaks into pieces of varying lengths, and these segments frequently withdraw into two distinct masses before the cell has started to constrict. More often, however, the chromatin is constricted in two with the cell (figs. 30, 31), and it is nothing unusual to see strands extending some distance into each daughter cell when the cells are connected only by a narrow isthmus (fig. 32). Two granules of chromatin occur regularly at the poles of the dividing cell at the maximum distance from the plane of constriction (figs. 30, 31, 32). These granules occupy these definite positions too often for this arrangement to be due to a mere fortuitous placing of waste chromatin, though what function is served is not at all clear. In the majority of cases the segments of the spireme in newly divided cells lie alongside the new wall formed by the constriction (fig. 33). If any spindle fibers were present in this division, the technic used caused them to be dissolved, as no traces of them were found. The evidence at present indicates that a typical resting stage is not necessary between successive cell divisions especially if the conditions are just right for rapid growth.

The dividing cells studied came from an agar culture in Chodat-Grintzesco solution. For a culture to thrive it must not be in a liquid medium or kept in the dark. No organic energy-yielding compound was necessary for rapid growth.

In searching for mitosis in a primitive or degenerate plant, the investigator is exposed to the danger which beset the late centrosome hunters, of mistaking a chance resemblance for a homologue. The eccentrically placed globule seems certainly to be chromatin, and whether we call it a nucleus or a nucleolus depends upon the relative flexibility with which we use these terms. In regard to its fragmentation it resembles the nucleolus, though if it is the nucleolus it contains all of the chromatin at this stage, which is not typical. The amount of chromatin apparently increases greatly as the fragmentation progresses, and this increase is too great to be explained by the increase in the precipitation of the stain on the increased surface exposed. Some of the stainable material may come from the fragmented pyrenoid. The chromatin is arranged in a typical spireme, which breaks up into segments of diverse sizes which may safely be considered as analogues of chromosomes. There is no lining up on an equatorial plane or any indication of the segments splitting and having their halves drawn to opposite poles. This method of nuclear division may be recorded as mitotic, but the mitosis is quite primitive and of an exceptional kind.

The bearing of this method of nuclear division upon the systematic position of Porphyridium must be uncertain until more is known of the nuclear history of the Bangiaceae. The resting stage is certainly unlike anything known in the Myxophyceae, though the later stages show a certain resemblance to this group. The whole process is a bit too primitive for the Chlorophyceae. In regard to its other characteristics Porphyridium resembles the Bangiaceae, and it would be best to keep it in this group.

The authors wish to thank Dr. W. R. Taylor for the material he supplied.

SUMMARY

1. Divergent results of the various investigators are probably due to their working with plants at different stages of growth rather than to their working on plants of different species.

2. The jelly is homogeneous only after a prolonged period of inactivity of the plant. In growing material the cells are born on gelatinous stalks.

3. The chromatophore is star-shaped only in the resting stages. Its red coloring matter can be extracted, leaving it green.

4. Porphyridium has a distinct, easily stainable, centrally located pyrenoid, which is generally spheroidal though sometimes ring-shaped.

5. In the resting stage Porphyridium has a single eccentric globule of chromatin homologous to a nucleus or nucleolus. Nuclear division is crudely mitotic.

6. Porphyridium had best be kept in the Bangiaceae at present.

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EXPLANATION OF PLATES

PLATE XX

These photographs were taken with a Gordon's photomicro camera. In figure 1, a Zeiss 2 mm. water-immersion objective and a Bausch and Lomb no. 10 eye-piece were used; in figures 2–12, a Zeiss 1.5 mm. oil-immersion objective and a Zeiss compensating ocular no. 6. The tube length was 160 mm. Magnification, × 400.

FIG. 1. Living cells showing cell division.

FIG. 2. Fixed in picric acid and stained in Heidenhain's haematoxylin.

FIG. 3. Same as figure 2.

FIG. 4. Fixed over a water bath with picric acid and stained with anilin-gentian violet.

FIG. 5. Fixed and mordanted with pyrogallic acid-ferrous sulphate, stained with anilin-gentian violet.

FIG. 6. Fixed as in figure 5. Not stained.

FIG. 7. Fixed as in figure 5. Stained with anilin-safranin.

FIG. 8. Stained by Van Ermengen's osmic acid-silver nitrate process.

FIG. 9. Fixed with tannic acid, stained with anilin-gentian violet.

FIG. 10. Fixed with a solution of pyrogallic acid and ferric bromide in ether, stained with safranin.

FIG. 11. Same as figure 10.

FIG. 12. Fixed in 1 part formalin and 1 part saturated solution of gentian violet in 95% alcohol, counterstained in Heidenhain's haematoxylin.

PLATE XXI

FIGS. 13–17. Living material showing increase in size and cell division. × 1070.

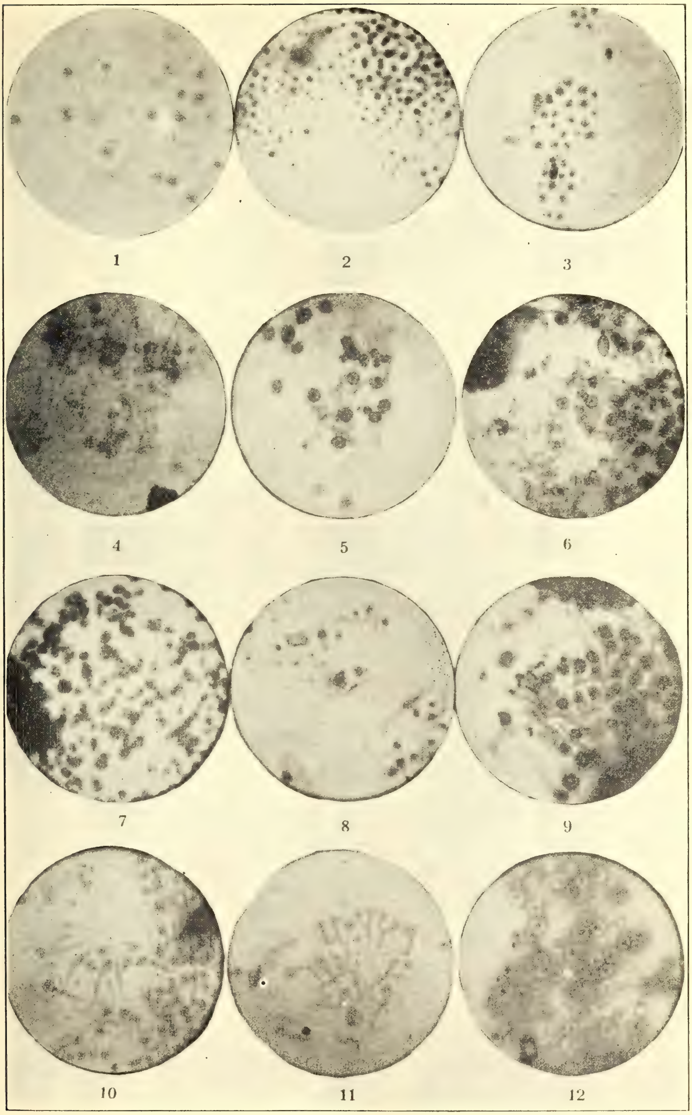
FIG. 18. Resting stage. × 1600.

FIGS. 19–27. Stages showing fragmentation of chromatin and changes in pyrenoid. × 1600.

FIGS. 28–34. Stages showing nuclear and cell division. × 1600.

FIGS. 35–38. Stages showing reassembling of chromatin. × 1600.

FIG. 39. Cells connected by stalks. × 1600.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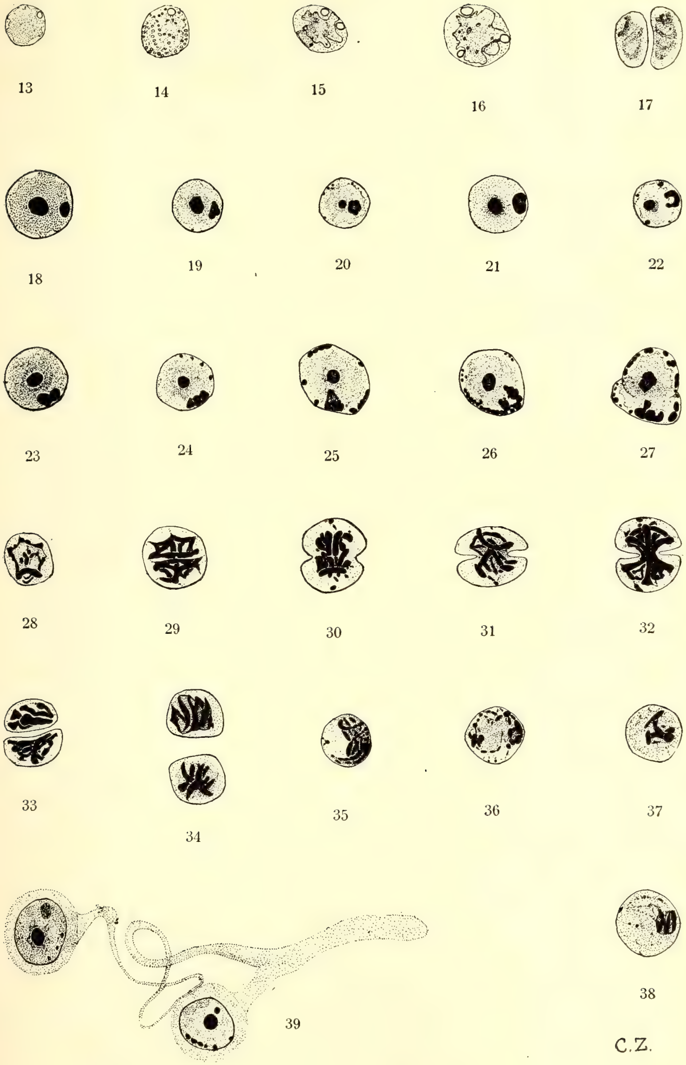
LEWIS AND ZIRKLE: CYTOLOGY AND SYSTEMATIC POSITION OF PORPHYRIDIUM.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LEWIS AND ZIRKLE: CYTOLOGY AND SYSTEMATIC POSITION OF PORPHYRIDIUM.

SOMATIC CHROMOSOMES IN TRADESCANTIA

Lester W. Sharp

Introduction

In 1913 the writer published the results of a study of chromosome behavior in the somatic cells of *Vicia faba*, the principal conclusion reached being that the splitting of the chromosomes is a phenomenon of the prophase. This view was directly opposed to that of Lundegårdh (1910, 1912), Fraser and Snell (1911), and Miss Digby (1910), who had contended that the telophasic alveolation of the chromosomes represents a splitting, and that the chromosomes remain through the ensuing resting stages as double structures. It was pointed out by the writer that this latter interpretation is rendered untenable by two lines of evidence which appear when the various transformations of the chromatin are minutely examined: first, the telophasic alveolation is very irregular and transforms each chromosome into an alveolar and then into a reticular structure which is in no true sense double, although chance arrangement of the vacuoles may often make it appear so; and second, the reticular chromosome, after separating from its fellows in the common reticulum during prophase, gives rise in a peculiar manner to a single (*i.e.*, not double) slender thread in which vacuoles, all or nearly all of them new, appear and develop into the true split.

The theory of telophasic splitting has been restated by Fraser (1914), Digby (1914, 1919), and Nothnagel (1916), who have employed it in the attempt to solve the problem of the heterotypic prophase. This point is one of fundamental importance, and will be taken up in some detail in the discussion.

The present study of the somatic chromosomes of *Tradescantia virginiana* L. was undertaken not only to test the writer's position with respect to the time of chromosome splitting in somatic mitosis, but also, by determining more precisely the nature of the transformation of the chromosomes in the somatic telophase, to ascertain to what extent, if at all, this transformation will aid in the interpretation of the heterotypic prophase. Essentially the same methods were employed as in the former investigation of Vicia. Although the behavior of the chromatin in the two cases is strikingly similar, Tradescantia, so far as could be judged from the preparations obtained, proved to be inferior to Vicia for a study of the late prophases; for the analysis of the critical stages of the telophase and early prophase, however, it turned out to be quite superior, many exceptionally clear figures being obtained. Root tips alone were used.

Among previous works dealing with Tradescantia may be mentioned those of Strasburger (1900), Miyake (1905), and Farmer and Shove (1905). The first two papers treat of the maturation mitoses. Both Strasburger, in his figures 97–100, and Miyake, in his figures 152 and 153, suggest a process of alveolation in the chromosomes during the heterotypic telophase. Farmer and Shove give an account of both the somatic and the maturation mitoses. They speak of a "vesiculation" of the chromosomes in the somatic telophase, the chromatin becoming a "cloud of fine granules through the linin band"; and describe "broad band-like areas" with the chromatin in a dense "granular aggregation" during the early telophase. These conditions are faintly suggested in their figures 2, *2a*, 20, and 21. But in none of these investigations have the changes undergone by the chromatin been followed closely enough to afford evidence on the time of chromosome splitting, or on the precise manner of the transformation of the chromosomes into the resting reticulum and of the latter into chromosomes.

DESCRIPTION

In order to give an uninterrupted account of the history of the chromatin through the critical stages—the telophasic transformation of the chromosomes into the resting reticulum and the subsequent condensation of the latter into chromosomes—the description will begin with the metaphase.

Metaphase and anaphase. As is usually the case with long chromosomes, those of Tradescantia are inserted on the spindle by their middle points. Six of them are thus shown in figure 1 (a portion of the chromosome on the right has been cut away). Since no detailed comparison of all the chromosomes of the group has been made, it is not known whether or not this mode of insertion is an invariable one. The free ends of the chromosomes extend out in various directions, but most commonly lie more or less parallel to the axis of the mitotic figure. As Farmer and Shove also observe, the doubleness of the chromosomes reaches its maximum distinctness at this time.

Because of their mode of attachment to the spindle, the chromosomes take the form of V's as they move toward the poles at anaphase (figs. 2, 3). The mottled appearance shown by the chromosomes appears to be due very largely to their uneven contour, though unequal density of the chromatin in various portions of the chromosome may be partly responsible. Nothing which can be called internal granules or chromomeres has been distinguished at this stage, and only occasionally do any vacuoles make their appearance so early. As they reach the poles the chromosomes become much shortened and thickened, and contract into two dense groups in which the limits of the chromosomes can be made out only with considerable difficulty. The new cell plate now begins to be differentiated on the fibers between the two groups.

Telophase. After remaining in close contact for a short time the chromosomes begin to separate from one another, and as they do so they cohere at various points where their substance becomes drawn out to form anastomoses (fig. 5). Although it is possible that some of the anastomoses, which become very numerous in later stages, may originate after the manner of pseudopodia, it is clear that the earlier ones must be formed mainly by the coherence of the viscid substance of neighboring chromosomes originally in contact. Meanwhile the karyolymph has begun to form, the nuclear membrane differentiating where it comes in contact with the cytoplasm.

The telophasic alveolation or vacuolation of the chromosomes begins at about the time the latter begin to separate as above described. The vacuoles first appear within the chromosomes as somewhat obscure though rather sharply limited regions of circular or oval form (fig. 5). They develop not only along the axis of the chromosome but also near or against its periphery; in fact, an inspection of the figures shows that they may occur in almost every conceivable position and with no regular arrangement with respect to each other. At a slightly later stage they become clearer and more numerous (fig. 6).

This variety and irregularity in the arrangement of the vacuoles calls for special emphasis, because of the fact that the writers named in the first paragraph of this paper have interpreted the telophasic alveolation as a splitting, the chromosomes from this stage onward being consequently regarded as double. Attention is therefore directed to the conditions illustrated in figures 6–9. It is noticeable that the vacuoles may nearly all be along the margin of the chromosome (right edge of upper nucleus in figure 6), and also that two or three may lie side by side across the width of the chromosome (left edge of same nucleus). In figure 7 are shown two chromosomes in which the latter condition is especially pronounced; here it is manifestly impossible to speak of a split. Transverse sections of the chromosomes at these stages are particularly instructive (fig. 9). Such a section passing through a region where there is but one large vacuole more or less centrally placed has an appearance represented in figure 9*a*. A chromosome with a series of such central vacuoles would appear double if viewed from the side. This, however, is only a special case of a more general condition. Figures 9*b*–9*f* show sections passing through regions occupied by several vacuoles side by side, as in the chromosomes of figure 7 and those at the left in figure 6. It would seem to require no further argument to show that the chromosomes during these and the later telophase stages are not split ribbons or threads, but are irregularly alveolated cylinders; and that they can no more be called "double" than triple or quadruple.

The above described changes continue, gradually transforming the alveolated chromosomes with their anastomoses into a common reticulum. An examination of figures 10–13 will serve better than a written description to make clear the manner in which this transformation is accomplished. The whole nucleus enlarges, nucleoli appear, the anastomoses lengthen and apparently become more numerous, and the alveolation of the chromosomes becomes more complete (fig. 10). The chromosomes at this time usually show a distinct polarity in their arrangement, as represented semidiagrammatically in figure 8. As the vacuoles within the chromosome increase in size and number, their boundaries at the margin of the chromosome break down, allowing them to become continuous with the nuclear cavity. In this way the alveolar or vacuolate condition passes into a reticular one; each chromosome becomes an irregular netlike structure which is in no sense double. All of them together, with their connecting anastomoses, constitute the common reticulum of the late telophase and interphase or resting stages. The limits of the constituent chromosomes remain visible until a comparatively late stage (fig. 11).

Interphase and Rest. The degree to which the telophasic transformation is carried varies considerably in different nuclei, the amount of change being correlated with the rapidity with which the mitoses succeed one another. In the most active part of the root meristem it seems evident that the transformation may go no further than the stage represented in figure 11, the prophasic changes of the next mitosis setting in at once. In such an event the chromatin passes directly from the stage of figure 11 (telophase) to that of figure 14 (prophase): the anastomoses connecting the reticulate chromosomes begin to break down while the chromosomes are yet distinguishable, so that there is no time between the successive mitoses at which the limits of the chromosomes cannot be seen. It is plain that in such cases the structural identity of the chromosomes is not lost during the interphase.

The interphase condition most commonly found in the root meristem is that shown in figure 12. Here the telophasic transformation has been carried much further; the anastomoses for the most part cannot be distinguished from the other fine strands of the reticulate chromosomes, and the limits of the chromosomes cannot be made out with any certainty. Here and there are lighter regions which probably represent boundaries between the constituent chromosomes, and if the prophasic changes were to begin at this time the reticulum would almost surely break down along these lines. The chromatin may apparently continue in this state for some time, so it may properly be said to be in the "resting" condition.

In older tissue, but only rarely in the root meristem, the telophasic changes continue until the nucleus has the structure shown in figure 13. The chromosomes, all visible traces of whose limits have now been lost, form a common chromatic reticulum of very fine texture. Such a reticulum is ordinarily described as being made up of "granules of chromatin carried on a supporting network," and so it surely appears if not sharply stained or if viewed through lenses of insufficient resolving power. But careful examination, together with a comparison of the successive stages of the telophase, lead to a different interpretation. Since the structure of the fine reticulum is the direct outgrowth of the progressive transformation shown in figures 5–12, it seems more probable that the "chromatin granules" are merely the heavier portions of the alveolated and reticulate chromosomes, and that the lighter "supporting network" consists simply of the thinner portions of the same together with the delicate anastomoses. As pointed out in the case of Vicia, the finer strands stain much less deeply than the coarser portions, so that one easily gains the impression of separate chromatin granules connected by delicate threads of another material. But in a tapering strand the color grows gradually deeper in passing from the thinner to the thicker portion, a fact which indicates that the reticulum consists of but a single substance, or, more probably, that the chromatin substance is very fluid in consistency and free to diffuse about within the other material which composes the framework, as suggested by Grégoire (1906). Although much evidence which has been brought forward by various workers indicates the presence of two principal and morphologically distinct elements in the nuclear reticulum, it is nevertheless probably true that the above interpretation will apply to many accounts describing autonomous chromatin units on a supporting achromatic network.

Prophase. As the prophasic changes begin, the reticulum becomes somewhat coarser and commences to break up into irregular band-like portions (fig. 14). As pointed out in the preceding section, the telophasic changes in rapidly multiplying cells may go no further than the stage represented in figure 11, where the limits of the chromosomes can easily be made out. If, now, such a nucleus should enter upon the prophase, there can be little doubt that it would be along the lines of chromosome union that the reticulum would break down, since along these lines are the delicate anastomoses, which would be the first to give way as the band-like portions begin to condense. A reticulum in which the telophasic transformation has been carried further, as in figure 12, also breaks down along its lighter zones. From what has been seen in the case of figure 11, it seems evident that these zones for the most part represent the interchromosomal spaces, so that here also argument may be made for the structural continuity of the individual chromosomes through the interphase or resting stage. In the case of a nucleus with such a fine and uniform reticulum as that of figure 13, it is manifestly impossible to determine by direct observation whether or not the lines of prophasic separation coincide with those of the preceding telophasic union. The evidence for the structural individuality of the chromosomes must here be indirect, and such indirect evidence is afforded by the many known instances in which the chromosomes in successive mitoses, although lost to view during the resting stages, not only remain constant in number but maintain constant differences in size and shape. Additional evidence is found in the fact that in cells dividing repeatedly in one plane, as in the root meristem, the separate bands or chromosomes appear in the prophase with the same orientation as that shown by the chromosomes as they form the reticulum during the telophase (compare figs. 11 and 14).

The separating portions of the reticulum, each of them representing a chromosome, continue to condense, and the anastomoses between them become further broken down, so that they soon stand out with great distinctness (fig. 15). Since the early prophasic changes are in many respects simply the reverse of those occurring in the telophase, the structure of the chromosomes at these two stages is almost precisely the same. Vacuoles, or spaces enclosed during the condensation of the reticulum, may be found in all positions, median and peripheral, while large cavities open to the exterior on all sides. Transverse sections of chromosomes in this condition are shown in figure 16. It is perfectly evident that at this stage of the prophase, just as in the telophase, each chromosome is simply an irregular alveolar-reticulate cylinder, and has the form neither of a split thread or ribbon nor of a ladder-like structure characteristic of the incompletely split chromosomes of the later prophases. At the stage shown in figures 15 and 16 the chromosomes are in no sense double; the split marking the line of separation at the next metaphase has not yet appeared.

Each alveolar-reticulate chromosome now becomes transformed in a peculiar manner into a *single* (*i.e.*, not double) slender thread. Of all the morphological changes undergone by the chromosomes during mitosis this is one of the most difficult to observe and interpret. The staining must be particularly sharp to bring it out properly, and it is probable that it is passed through with considerable rapidity. These facts account in part for the absence of these stages from the descriptions of mitosis given by many investigators, and consequently for misinterpretations of the telophasic and early prophasic changes with respect to the splitting of the chromosomes. The transformation process, which is in progress in the nucleus shown in figure 17, is as follows.

As the chromatic material becomes increasingly condensed, the more slender strands, like the anastomoses at an earlier stage, and also the thinner walls bounding the peripherally situated enclosed spaces, become broken down, leaving the heavier portions of the chromosome in the form of a very irregular zigzag thread of uneven thickness. Various stages in this process are shown in figure 18. At the points marked *a* the dissolution of the finer portions can be seen occurring, and the reason for the crooked and lumpy appearance of the newly formed slender thread is apparent. In many cases the arrangement of the open spaces is such that the chromatic thread has a roughly spiral aspect, but in view of the relation it bears to the reticulate stages of the earlier prophases it can hardly be said to arise endogenously within the chromosome as some workers have maintained. The crooked thread at once begins to straighten out; this change in shape is associated with an equalization of the chromatic material, so that the thread gradually becomes more uniform in diameter. Here and there in the heaviest portions a few small vacuoles may for a time persist, but for considerable distances the thread is completely without them.

The true split now develops in the slender threads. Almost as soon as a portion of a thread becomes sufficiently equalized a number of new openings appear, and it seems highly probable that they are the outgrowth of small vacuoles which are formed anew along the axis of the thread. Some of the openings elongate a little, making the thread clearly double for short distances (fig. 19); here for the first time a portion of a chromosome can be said to be truly double. It is a matter of extreme difficulty—indeed it is probably impossible—to tell certainly whether these small vacuoles and openings are all new prophasic developments or are in part retentions from the resting stage and hence from the preceding telophase. Soon after their formation the slender threads, so far as can be determined with the best optical equipment, are certainly single and devoid of vacuoles for long distances, whereas the vacuoles become very numerous later. After a comparison of many chromosomes in these stages the writer has concluded that in all probability a vacuole or open space is now and then retained from an earlier stage, but that the great majority of vacuoles and spaces which develop into a split in the slender thread are formed anew in the prophase.

As the vacuoles increase in number and enlarge into openings extending through the thread, the latter shortens and thickens, and takes the form of an irregular ladder-like structure (figs. 20, 21). A nucleus in this stage (fig. 22) has a superficial resemblance to one in the early prophase stages (fig. 15), and some writers have confused them, omitting the important stages which intervene. In figure 15 the chromosomes are in the form of irregular alveolar-reticulate cylinders, as shown by their cross section (fig. 16), whereas in figure 22 they exist as thread- or ribbon-like structures partially split by a series of median openings and are clearly double in cross section (see the free and cut ends in figures 21–23).

As already stated, Tradescantia appears to be less favorable for a study of the later prophases than Vicia. The open spaces in the chromosomes do not run together to form a continuous split so early as in the latter plant. The material of some of the cross pieces connecting the sides of the partially split threads gradually flows to the two sides where it accumulates in the form of paired lumps simulating divided granules as fully described in the account of Vicia. Many of them, however, remain unchanged until a very late stage, so that even after the threads have become much shortened and thickened to form the conspicuous heavy spirem stages some of them may show the openings not yet developed into a complete split (figs. 22, 23). In other chromosomes the splitting process has gone further, making them almost completely double (at right in figs. 22 and 23). In nuclei of these stages it is not difficult to discover several free ends which are not due to the microtome knife, so that certainly at this time, and probably at earlier stages also, the chromosomes do not form a continuous spirem. On the other hand, it seems probable that some of the chromosomes may hang together end to end, since the number of ends which can be distinguished appears to be much smaller than the number which would be expected if all the chromosomes were free from one another.

The spindle now begins to differentiate in the cytoplasm, and the nuclear membrane contracts about the chromosomes. In the chromosomes the split now becomes complete, but as they continue to shorten and thicken their halves become so tightly pressed together that in many preparations they can scarcely be distinguished. As the contraction reaches its climax the nuclear membrane disappears (fig. 24), and the chromosomes, after loosening up as an irregular group, rapidly become arranged on the spindle with their halves clearly evident (fig. 1).

Discussion

A number of the features of somatic mitosis as the writer has found them have been compared with the results of other investigators in the paper on Vicia (1913) and need not be reconsidered here. In the present discussion attention will be limited to three important points: the time of chromosome splitting, the method of splitting, and the bearing of the results of this study on certain interpretations of the heterotypic prophase.

Time of chromosome splitting. Because of the exact manner in which the telophasic and prophasic changes are seen to occur when closely examined, the writer has contended that the definitive splitting of the chromosomes occurs in the prophase rather than in the telophase as several workers have urged. In the first place, the telophasic alveolation, as emphasized in the foregoing description, is a very irregular process, its result being the transformation of each chromosome into an irregular alveolar-reticulate structure showing nothing which can with any justice be called doubleness. After an inspection of the figures of longitudinal and cross sections of the telophase chromosomes (figs. 5–11) further argument on this point would seem to be superfluous. Secondly, the alveolar-reticulate bands into which the resting reticulum breaks down in early prophase, and which are probably in all cases continuous with the similar bands (chromosomes) of the preceding telophase, are not transformed directly into the split spirems of the later prophase, but give rise to single threads in which the definitive split is formed as the result of a process which appears to begin with the development of an axial series of new vacuoles. It has been shown that as these single threads are evolved most of the vacuoles and open spaces which had their origin in the preceding telophase, and which constitute the openings in the resting reticulum, become lost through the breaking down of their boundaries, so that the telophasic vacuoles, whether so situated as to make the chromosome double or not, take little or no part in the development of the definitive split.

It may here be questioned whether the vacuoles and open spaces which appear and split the thin prophasic thread may not be at least partly retentions from the preceding telophase, the split therefore being initiated in the telophase after all. This is a question which it has not been found possible to answer in many cases, since the changes in question occur in very minute structures which cannot be interpreted at all in any but the most favorable preparations. There can be no doubt, however, that the threads for considerable distances are actually single so far as the microscope will allow us to determine, and that many new vacuoles and open spaces develop where none were visible before. On the other hand it seems very probable that a few spaces of the earlier prophase, and hence of the preceding telophase, may occasionally persist in the heavier portions of the threads as they develop from the reticulate bands, such spaces if properly situated being incorporated in the later true split. But only in a very strained sense could such occasional vacuoles or spaces be said to constitute the initial stages of the split; their relation to it appears to be fortuitous rather than determinative.

It may also be questioned whether the single thread stage of the prophase, upon the importance of which the writer has insisted, is a phenomenon of general occurrence or is a special process peculiar to a few types of cells. To this question also no full answer can be given at present. It surely occurs in the root cells of Vicia and Tradescantia in spite of the fact that it does not appear in the descriptions given by other investigators of mitosis in these plants. It is also represented in Müller's (1911) figure 9 of *Najas marina*, and in the "spiral threads" figured by Bonnevie (1908, 1911, 1913) for Allium, Amphiuma, and Ascaris; by Wilson (1912) for certain insects; and by several other investigators. How much more widespread it may be cannot be stated, especially since the prophasic changes have been followed with sufficient care in so relatively few cases.

On the contrary, it is not impossible that in some forms the split spirem may develop directly from the alveolar-reticulate bands of the earlier prophase by a rearrangement of the vacuoles and openings to form a single median series as the structures become more slender, but the writer is not convinced that such a process has been demonstrated in any instance. Even if, for the sake of argument, it were assumed to occur, it would not necessarily follow that the telophasic vacuolation should be regarded as a splitting or that the chromosomes of the late telophase, resting stage, and early prophase should be regarded as double when the vacuoles and openings have such an arrangement as that described for Vicia and Tradescantia. Chromosomes in this condition are not double in any sense of the word, even though they contain open spaces which may later join with others to form a split. The chromosome can be said to be "double" or "split" only when its substance has been separated into two distinguishable portions, either by the rearrangement of the vacuoles and spaces as provisionally assumed, or, as is in all probability actually the case, by the formation of a median series of vacuoles and spaces which are for the most part if not entirely of prophasic origin. It may again be emphasized that in Tradescantia, as in Vicia, the alveolar-reticulate condition of the telophase and the early prophase does not pass directly into the split spirem stage as above provisionally assumed; but rather, by a peculiar process in which most if not all of the old vacuoles and openings are lost, the reticulate chromosome takes the form of a single thread in which the split then develops anew. In these forms the chromosome split, whether entirely new or partially built of vacuoles of earlier origin, cannot be regarded as anything but a prophasic development.

Method of chromosome splitting. Much interest centers about the exact manner in which the chromosomes undergo splitting because of its great importance in connection with current theories of the mechanism of heredity. The early view that the splitting of the chromosomes is primarily a division of a series of smaller units which it contains has been widely accepted, especially by those whose investigations have been concerned with the cytological aspects of the problem of inheritance. The hypothesis which postulates the existence of small units of inheritance, or genes, within the chromosomes has been of incalculable value in organizing the data of genetics, and new evidence constantly adds to the probability that such units are not purely conceptual ones. But it must be said that this evidence is genetical rather than cytological in nature. Although many cytologists have regarded the visible chromatin accumulations or granules as such units and have figured their division, others have found it increasingly difficult to see in these chromomeres anything significant in this connection. For the most part they seem to be quite inconstant in number and disappointingly irregular in behavior.

In the somatic cells of Tradescantia, as in Vicia, chromosome splitting seems to be initiated by a series of axial vacuoles which quickly develop into openings through the homogeneous chromatin thread, and not by the division of chromatic granules supported by the thread. As the openings become more numerous the chromosome takes the form of a ladder, the rounds being represented by the material between the successive openings (fig. 21). As the rounds or cross pieces gradually become thinner and finally break at the middle, thus completing the split, their material accumulates in two small lumps opposite each other in the two halves of the chromosome. The "paired granules" or "divided granules" described by many workers are without doubt to be explained in this manner. Such being their mode of origin, it is difficult to assign to them the rank of morphological units, or to attribute to them any specialized function in the hereditary process. In the microsporocytes of Vicia, however, the writer has observed chromatic granules of a much more definite character, but is not prepared to make any statement with regard to their significance.

The view that such granules do have some special significance is strongly favored by Wenrich's (1916) striking observations on Phrynotettix. Not only do the granules or chromomeres of a given member of the chromosome group in this form have relatively constant sizes and positions, but they also show close correspondence in the two members of a conjugating homologous pair. This is precisely the type of chromosome organization called for by our most promising theory of the cytological mechanism of heredity, which it is hoped will find further verification in additional observations as carefully made as those of Wenrich.

Bearing on problem of chromosome reduction. As stated in the introduction, the theory of telophasic splitting has been incorporated in an interpretation placed upon the reduction phenomena by Fraser (1914), Digby (1914, 1919), Nothnagel (1916), and certain other writers. The split seen in the early heterotypic prophase is said to have its origin in the telophase of the last premeiotic division, each chromosome persisting through the intervening resting stage in the double condition. It is consequently held, as fully stated by Digby (1919) in her account of the archesporial and meiotic phases in Osmunda, that the lateral pairing of slender threads in the heterotypic prophase, which a large school of cytologists has regarded as a conjugation of entire chromosomes, is in reality only the reassociation of the two halves of one chromosome which has been split in the preceding telophase. Such a reassociation is thought to occur in every prophase, somatic and meiotic, since these workers regard chromosome splitting as universally a telophasic phenomenon. The split thus thought to form in the premeiotic telophase functions in the homoeotypic mitosis; the latter mitosis is therefore looked upon as a continuation of the premeiotic division, the heterotypic mitosis being an interpolated process bringing about reduction. Not only does this premeiotic split reappear in the anaphase of the heterotypic mitosis to function in the homoeotypic, but a new split developing in the heterotypic telophase, after being temporarily obscured, functions in the post-homoeotypic division.

As the writer (1920) has pointed out in a review of Digby's contribution, the above outlined theory of reduction has certain advantages, for "it allows one interpretation to be placed upon the double spirem in both somatic and heteroptyic prophases, . . . and it also helps to explain the sudden appearance of the split for the second maturation mitosis in the anaphase of the first."

But can it be said that the chromosomes undergo splitting in the last premeiotic telophase and remain double through the ensuing resting stage? The writer believes that it has been shown in the case of ordinary somatic mitosis in Vicia and Tradescantia that the telophasic alveolation is in no sense a split. This conclusion rests upon the facts brought out in a detailed analysis of the telophasic changes themselves, and upon the fact that the early prophasic reticulate condition, which all grant is continuous with that of the preceding telophase, does not pass directly into the double spirems, but gives rise to single threads in which a new split develops, entirely or mainly by a new vacuolation, making the threads really double for the first time. This being true, it follows that the chromosomes at the beginning of the heterotypic prophase are *single* (although alveolar-reticulate), unless, indeed, the premeiotic telophase differs fundamentally from other somatic telophases, which is not supposed to be the case. Consequently, if it is argued that the doubleness of the spirem in the heterotypic prophase is due to a splitting and not to a conjugation, it must be done upon other grounds; the principle of the telophasic split is evidently a false premise.

From the above considerations it appears that chromosome behavior during the somatic telophase, instead of giving the key to the reduction process, shows rather that the solution of this perplexing problem must be reached mainly through a more refined analysis of those changes in the heterotypic prophase itself which have led so many observers to conclude that the association of chromatic threads at that stage represents the conjugation of entire chromosomes which separate at the first maturation mitosis.

Summary

The principal results of the present study of Tradescantia may be summarized as follows:

1. During telophase the chromosomes become transformed by a process of irregular vacuolation into alveolar-reticulate structures which together make up the resting reticulum. In rapidly multiplying cells the visible limits of the constituent chromosomes may not be entirely lost between successive mitoses.

2. In prophase the constituent reticulate chromosomes separate from one another through the breaking down of their connecting anastomoses; each gives rise to a single slender chromatic thread in which the definitive split develops as a new formation, though old vacuoles may occasionally be incorporated in it.

3. The telophasic vacuolation cannot be interpreted as a splitting of the chromosomes; the chromosomes are therefore not double during the resting stages. The splitting of the chromosomes is a phenomenon of the prophase.

4. No direct evidence has been found favoring the view that the splitting of the chromosomes is primarily a division of smaller chromatic units or chromomeres.

5. Interpretations of the phenomena of the heterotypic prophase are unsound in so far as they rest upon the assumption of telophasic splitting in somatic cells.

Cornell University

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EXPLANATION OF PLATES

PLATE XXII

All figures except no. 8 were drawn at the level of the table with the aid of an Abbé camera lucida under a Zeiss apochromatic objective, 2 mm. N.A. 1.40, with compensating ocular 18. They have been reduced one-half in reproduction and now show a magnification of 1900 diameters. Fig. 8, × 950.

FIG. 1. Metaphase: chromosomes attached to spindle by middle points.

FIG. 2. Anaphase: daughter chromosomes separating.

Fig. 3. Later stage: daughter chromosomes passing to poles.

Fig. 4. Late anaphase: chromosomes massing at poles.

Fig. 5. Telophase: vacuoles appearing within chromosomes; anastomoses present.

Fig. 6. Slightly later stage; note arrangement of vacuoles.

Fig. 7. Two telophase chromosomes, showing irregularity of vacuolation.

Fig. 8. Telophase, showing polarized arrangement of chromosomes (camera lucida sketch).

Fig. 9. Cross sections of telophase chromosomes, showing the same.

Fig. 10. Later telophase: nucleus larger and nucleoli present.

Fig. 11. Late telophase: limits of chromosomes still visible.

Fig. 12. Interphase or resting stage.

Plate XXIII

Fig. 13. Resting stage in which telophasic changes have gone further.

Fig. 14. Early prophase: reticulum breaking up into constituent chromosomes.

Fig. 15. Later stage: chromosomes further separated and more condensed.

Fig. 16. Cross sections of chromosomes like those of figure 15.

Fig. 17. Prophase nucleus with most of its chromosomes in single thread stage.

Fig. 18. Chromosomes in single thread stage; development of this condition from reticulate stage shown at *a*.

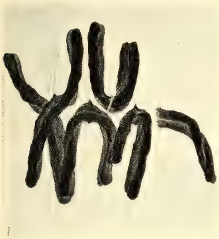
Fig. 19. Splitting of single threads.

Figs. 20, 21. Later stages: vacuoles and openings more numerous; chromosomes thicker.

Fig. 22. Prophase nucleus with some chromosomes partially and some completely split.

Fig. 23. Later stage: chromosomes heavier but not completely split.

Fig. 24. Late prophase: chromosomes swollen and split obscured; nuclear membrane has disappeared and spindle is developing.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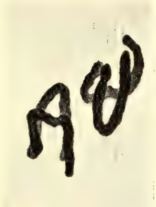
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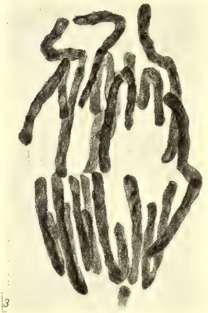
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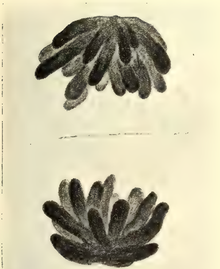
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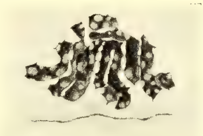
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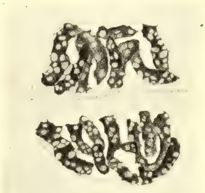
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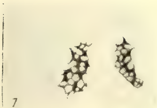
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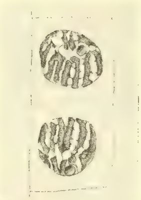
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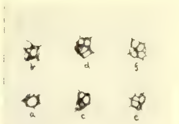

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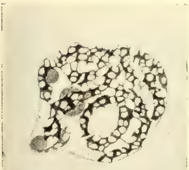
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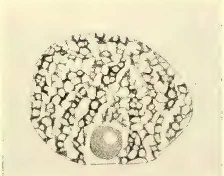
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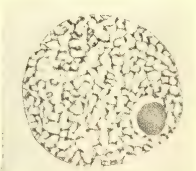
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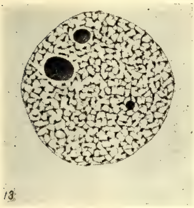
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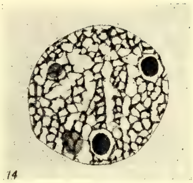
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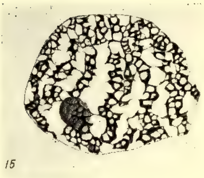
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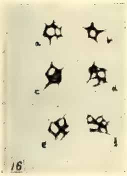
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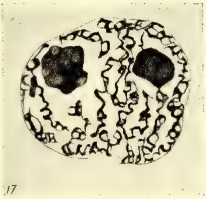
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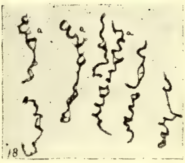
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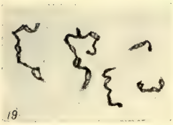
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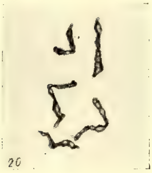
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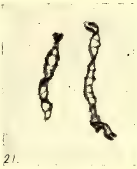
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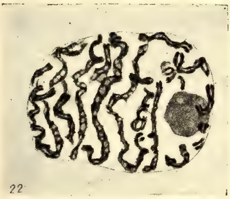
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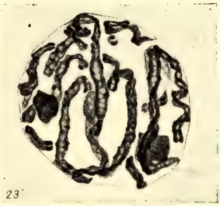
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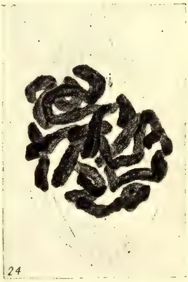
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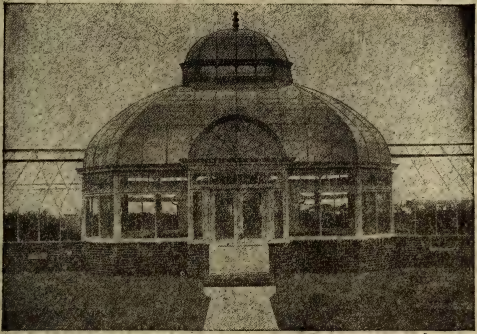

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THE CAMBIUM AND ITS DERIVATIVE TISSUES
II. SIZE VARIATIONS OF CAMBIAL INITIALS IN GYMNOSPERMS AND ANGIOSPERMS

I. W. Bailey

Introduction

Much has been written during the last fifty years concerning the relations between cell size, and body size, nuclear size, chromosomal number, and chromosomal mass. One group of botanists and zoologists, including such classical writers as Sachs (1893), Driesch (1898, 1900), and Boveri (1904), maintain that the size of the cells in specific organs or organisms remains constant regardless of variations in growth or stature, whereas another group hold that cell number rather than cell size is fixed. A second controversy revolves around the question whether the nucleo-cytoplasmic relation is a constant or a self-regulating ratio, and, more recently, whether dwarf and giant mutants are produced by changes in the number or in the size of chromosomes.

Many of the discrepancies in the conclusions of these writers appear to be due to an intensive study of a particular tissue, organism, or stage in ontogeny without reference to what may occur in other tissues, organisms, or developmental stages. Levi (1906) has shown that in mammals the size variations of epithelial and gland cells—elements which continue to divide throughout life—are insignificant, whereas such highly differentiated cells as nerve fibers, lens fibers, muscle fibers, and ganglion cells tend to be considerably larger in large animals than in small ones. Thus, the necessity for *extensive* preliminary, comparative investigations in selecting material for *intensive* experimental research, and to serve as checks upon excessive generalization from limited induction, is well illustrated by the literature dealing with body size and cell size.

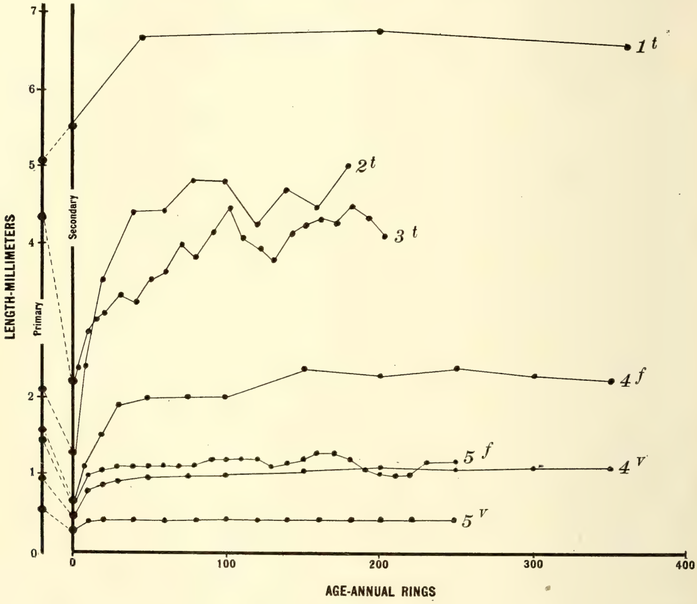
In the first investigation of this series[1] an attempt was made to determine, by means of an extensive reconnaissance survey, what are some of the more fundamental types of size variations that occur in the tracheary

---

[1] Bailey, I. W., and Tupper, W. W. Size variation in tracheary cells: I. A comparison between the secondary xylems of vascular cryptogams, gymnosperms and angiosperms. Proc. Amer. Acad. Arts and Sci. **54**: 149–204. 1918.

[The **Journal** for October (**7**: 305–354) was issued November 5, 1920.]

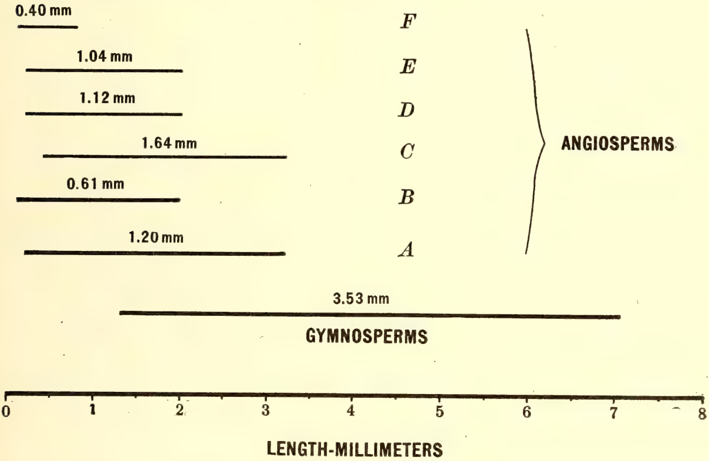
cells of the secondary xylem of vascular plants. The elements were found to fluctuate considerably in length in different parts of an organ or plant, in individuals grown under different environmental conditions, and in different groups of the Siphonogama. As shown in text figure 1, the average length of the tracheary cells, in a given radius and at a particular height in the stem of an arborescent dicotyledon or gymnosperm, is not constant in succeeding annual rings, but tends to increase rapidly for a period of years and subsequently to fluctuate more or less above and below a certain general level. This length-on-age curve varies in different portions of the stem and in plants grown under different environmental influences. In normal forest trees, its crest tends to be higher in the "clear length" of the stem and lower in the crown, in the stump, and in proximity to burls, severe injuries, and other disturbing factors. Although these somatic variations, due to physiological and ecological factors, are so varied and extensive as to render



Text Fig. 1. Curves showing variations in average length of tracheary cells in passing from the innermost to the outermost secondary xylem of the stem. Average lengths of primary tracheary elements shown for comparison. 1, cycad; 2, conifer; 3, vesselless dicotyledon; 4, dicotyledon with primitive vessels; 5, dicotyledon with highly differentiated vessels. *t*, tracheids; *f*, fiber tracheids; *v*, vessel-segments. Modified from Bailey and Tupper.

difficult the isolation of germinal fluctuations in a limited number of closely related plants, the study of a wide series of Siphonogama reveals striking differences in the size of the tracheary cells in different groups of plants. For example, the average length of the tracheids in the outer rings of the secondary xylem of 152 gymnosperms was 3.53 ± 0.07 mm. (SD = 1.25 ± 0.05 mm.); whereas in comparable material of 275 dicotyledons, from 31 orders and 118 families, the mean length of the fiber tracheids[2] and vessel-segments was 1.20 ± 0.02 mm. (SD = 0.50 ± 0.01 mm.) and 0.61 ± 0.02 mm. (SD = 0.41 ± 0.01 mm.) respectively (text fig. 2).

The reduced length of the tracheary elements in the secondary xylem of dicotyledons appears to be closely correlated with the development and differentiation of vessels. This is indicated, not only by the striking general contrast between the sizes of the tracheary elements in plants which have vessels (Gnetales, dicotyledons) and in those which are devoid of them (vascular cryptogams, gymnosperms, vesselless Trochodendraceae, and



TEXT FIG. 2. Limits of variability of average lengths of tracheids in the older wood of 152 gymnosperms contrasted with the limits of variability of (*A*) average lengths of fiber tracheids in older wood of 275 miscellaneous dicotyledons, (*B*) average lengths of vessel-segments in 275 miscellaneous dicotyls, (*C*) average lengths of fiber tracheids in older wood of 53 dicotyls having primitive vessels, (*D*) average lengths of vessel-segments in 53 primitive dicotyls, (*E*) average lengths of fiber tracheids in older wood of 169 dicotyls having highly specialized vessels, and (*F*) average lengths of vessel-segments in 169 specialized dicotyls. Mean of average lengths shown numerically.

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[2] Using this term in a general sense to include tracheids, fiber tracheids, libriform fibers, and septate fibers, but excluding substitute fibers.

Magnoliaceae, text fig. 1), but also by the fact that the tracheary cells in the dicotyledons tend to shorten as the vessels become more and more highly specialized (text fig. 2).[3]

In all of the arborescent dicotyledons and gymnosperms, with the probable exception of the Cordaitales, Bennettitales, and Cycadales, the first formed tracheary cells of the secondary xylem are relatively small and are considerably shorter than the adjoining elements of the primary xylem (text fig. 1). This is in marked contrast to the conditions which appear to have prevailed in the stems of many of the lower vascular plants. In forms having relatively wide zones of primary wood, the innermost secondary tracheids resembled in size the outermost primary tracheids. It seems probable that in the evolution of the higher gymnosperms and dicotyledons, with reduction in the amount of primary xylem and with other changes in the innermost portion of the stele, there has been a concomitant shortening of the first formed elements of the secondary xylem.

The size of the cells in the secondary xylem is determined by (1) the size of the cambial initials, and by (2) changes that take place in their derivative cells during differentiation into tracheary elements. It is conceivable, therefore, that the variations in the size of the tracheary elements may be closely correlated with similar fluctuations in the size of the meristematic cells. It is also conceivable, however, that the cells of the lateral meristem are of relatively uniform size, as hypothesized by Strasburger (1893), Winkler (1916), and others, and that the differences in the size of tracheary cells are due entirely to changes, *e.g.*, expansion, division, etc., which occur during differentiation of the xylem. The present paper is devoted to a comparative study of the size variations of cambial initials and tracheary cells.

MATERIAL AND METHODS

There are two methods of determining the sizes of the cells in a given tissue: by measurements taken (1) from sections and (2) from macerations. Each method has certain inherent advantages and disadvantages. In macerations it is possible to isolate individual cells and measure their various dimensions, but it is necessary to allow for differences in breakage,

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[3] The secondary xylem of the Calamariales, Sphenophyllales, Lepidophytineae, Cycadofilices, and Gymnospermae, exclusive of the Gnetales, is comparatively homogeneous, and its tracheary cells are of a single generalized type, so-called tracheids. In the Gnetales and Dicotyledoneae specialization or "division of labor" appears to have occurred among these cells. Certain vertical series of tracheids have become modified and function principally in conducting liquids, whereas others have assumed a mechanical rôle. As the vessels of the dicotyledons become more and more highly differentiated, their segments change their shape and structure and lose their resemblance to tracheids. At the same time, the surrounding tracheary elements tend to take on a more fiber-like structure, their pits becoming vestigial by the gradual disappearance of the bordering areas in the secondary walls.

shrinkage or contraction, etc. Of course, it is difficult to macerate the cambium and other soft tissues. The average length of vertically elongated elements may be obtained with a considerable degree of accuracy from longitudinal, tangential sections of tissues in which the elements are arranged in regular radial rows, *i.e.*, as in the cambium or xylem of gymnosperms. The lengths of the fiber tracheids and vessel-segments in most dicotyledons have to be obtained from macerations.

The measurements of the cells of conifers, recorded in the following table, were obtained from serial, tangential, longitudinal sections of the cambium and adjacent xylem, and were checked by measurements taken from macerations. In the case of the dicotyledons, the tabulated values were secured from tangential sections of the cambium and macerations of the outermost layer of the underlying xylem. The means are averages of fifty measurements, and their probable errors vary between 0.005 and 0.05 mm.

It is evident from these data that in Gingko and the Coniferae the length of the cambial initials closely resembles, but usually is slightly less than,[4] that of the tracheids of the last formed growth layer of the xylem. In the dicotyledons, on the other hand, the meristematic cells are in most cases considerably shorter than the fiber tracheids, but are of approximately the same length as the vessel-segments. However, they tend to be slightly shorter than the vessel-segments in species (Alnus, Euptelea, Myristica, Liquidambar, Rhizophora, Nyssa) having primitive types of vessels, and a little longer than these cells in plants having highly specialized conducting systems. Therefore, by allowing for a 5–10 percent error, it is possible to use the tracheids of gymnosperms and the vessel-segments of arborescent and fruticose dicotyledons as indexes of the approximate length of the cambial initials in these two important groups of the vascular plants.

The principal types of size (length) variations that occur in the tracheary cells of the secondary xylem are closely paralleled by similar fundamental fluctuations in the longitudinal dimension of cambial initials. Thus, these meristematic cells vary in different parts of a plant or organ, in individuals grown under different environmental conditions, and in different groups of the Siphonogama. They are relatively short in young shoots and twigs of Gingko and Coniferae, but during subsequent growth increase in length for a period of years until a certain size level has been attained, after which they fluctuate more or less in response to various physiological and environmental influences. In comparable material, the normal length-on-age curve for the cambial initials tends to be considerably lower and flatter in the dicotyledons than in the conifers, and in plants having highly differentiated vessels[5] than in those in which the conducting systems are relatively primitive (text fig. 3, page 363).

[4] Mischke's (1890) calculations of elongation are based upon an erroneous premise, as has been pointed out by Klinken (1914).

[5] In certain highly specialized dicotyledons the length of the short cambial initials, vessel-segments, and substitute fibers may remain constant or nearly constant during successive increases in the circumference of the stem, as suggested by Sanio (1873–74).

TABLE I.

Comparative Lengths of Tracheary and Meristematic Cells

GYMNOSPERMAE

	Cambial Initials			Tracheids		
	Max.	**Mean**	Min.	Max.	**Mean**	Min.
I. GINKGOALES						
1. Ginkgoaceae						
[4] *Ginkgo biloba* L.	3.0	2.2	1.4	2.9	2.2	1.4
II. CONIFERAE						
2. Taxaceae						
[6] *Taxus cuspidata* Sieb. and Zucc.	1.6	1.1	0.8	1.7	1.3	0.8
3. Pinaceae						
(*a*) Abieteae						
Pinus Strobus L.	4.0	3.2	2.3	4.6	3.4	2.2
Picea Abies (L.) Karst.	4.2	3.3	2.4	4.2	3.6	2.8
Larix decidua Mill.	5.0	4.0	2.5	5.4	4.2	2.7
[6] *Pseudotsuga taxifolia* (Lamb.) Britton.	1.6	1.2	0.7	1.8	1.2	0.9
[6] *Abies Nordmanniana* Spach.	1.5	1.1	0.7	1.8	1.2	1.0
[6] *Cedrus libani* Barrel.	2.6	2.0	1.2	2.7	2.1	1.3
[6] *Tsuga canadensis* (L.) Carr.	1.8	1.4	0.9	2.1	1.5	1.1
(*b*) Taxodieae						
[6] *Sciadopitys verticillata* Sieb. and Zucc.	1.6	1.2	0.7	1.6	1.3	1.0
[6] *Sequoia gigantea* Lindl. and Gord.	4.5	3.7	2.5	4.5	3.8	2.8
(*c*) Cupresseae						
[6] *Thuja occidentalis* L.	2.1	1.5	0.7	2.4	1.7	1.3
Juniperus virginiana L.	3.0	2.2	1.0	3.0	2.3	1.4

ANGIOSPERMAE-DICOTYLEDONEAE[7]

	Vessel-segments			Cambial Initials			Fiber Tracheids		
	Max.	**Mean**	Min.	Max.	**Mean**	Min.	Max.	**Mean**	Min.
A. ARCHICHLAMYDEAE									
I. SALICALES									
1. Salicaceae									
Populus sp.	0.70	0.50	0.19	0.66	0.49	0.35	1.19	0.90	0.61
II. JUGLANDALES									
2. Juglandaceae									
Carya glabra Sweet	0.63	0.43	0.20	0.70	0.56	0.40	1.69	1.13	0.65
Carya ovata (Mill.) C. Koch	0.55	0.51	0.47	0.60	0.52	0.42	1.69	1.30	0.96
III. FAGALES									
3. Betulaceae									
Alnus incana (L.) Moench	0.84	0.66	0.43	0.72	0.60	0.34	1.20	1.89	0.56
Betula populifolia Marsh.	1.17	0.89	0.65	1.16	0.94	0.70	1.60	1.31	0.91
4. Fagaceae									
Quercus alba L.	0.60	0.46	0.36	0.67	0.53	0.39	1.42	1.00	0.80
IV. URTICALES									
5. Ulmaceae									
Ulmus americana L.	0.59	0.33	0.21	0.47	0.35	0.27	1.96	1.53	1.12
V. RANALES									
6. Trochodendraceae									
Euptelea polyandra Sieb. and Zucc.	0.97	0.72	0.39	0.86	0.63	0.40	1.42	0.95	0.59
7. Annonaceae									
Annona reticulata L.	0.43	0.29	0.13	0.39	0.33	0.22	1.71	1.28	0.83

[6] Material obtained from small branches or young stems.

[7] Material obtained from stems of various ages.

TABLE I (*Continued*)

	Vessel-segments			Cambial Initials			Fiber Tracheids		
	Max.	**Mean**	Min.	Max.	**Mean**	Min.	Max.	**Mean**	Min.
Phaeanthus ebracteolatus Merr.	0.58	0.39	0.23	0.61	0.44	0.27	1.40	0.94	0.61
8. Myristicaceae									
Myristica philippensis Lam.	1.64	1.42	0.84	1.62	1.31	0.99	2.00	1.60	1.12
9. Lauraceae									
Litsea glutinosa C. R. Rob.	0.74	0.52	0.36	0.70	0.55	0.39	1.49	0.95	0.56
Sassafras officinale Nees and Eberm.	0.50	0.39	0.22	0.50	0.39	0.27	0.83	0.61	0.38
VI. Rosales									
10. Pittosporaceae									
Pittosporum pentandrum (Blanco) Merr.	0.90	0.66	0.29	1.01	0.80	0.56	1.22	0.99	0.76
11. Hamamelidaceae									
Liquidambar Styraciflua L.	1.39	0.76	0.41	0.98	0.70	0.40	1.75	0.96	0.67
12. Rosaceae									
Pyrus Malus L.	0.72	0.51	0.29	0.74	0.53	0.34	1.29	0.98	0.61
Prunus serotina Ehrh.	0.58	0.45	0.23	0.59	0.46	0.32	1.40	0.99	0.58
Pyrus sp.	0.80	0.57	0.44	0.77	0.66	0.52	1.17	0.92	0.59
13. Leguminosae									
Robinia Pseudo-Acacia L.	0.22	0.18	0.13	0.21	0.17	0.14	1.40	0.87	0.58
VII. Geraniales									
14. Burseraceae									
Canarium villosum F. Vill.	0.66	0.49	0.31	0.86	0.54	0.34	1.26	1.00	0.50
15. Meliaceae									
Xylocarpus granatum Koen. var.	0.47	0.36	0.13	0.67	0.37	0.23	1.39	0.97	0.61
16. Euphorbiaceae									
Excoecaria Agallocha L.	0.87	0.59	0.29	0.87	0.63	0.41	1.17	0.86	0.56
Glochidion littorale Bl.	1.28	0.90	0.36	1.21	1.04	0.72	1.84	1.52	0.92
VIII. Sapindales									
17. Anacardiaceae									
Anacardium occidentale L.	0.56	0.42	0.27	0.70	0.44	0.25	0.88	0.66	0.47
Buchanania arborea Bl.	0.63	0.41	0.29	0.61	0.41	0.27	1.17	0.97	0.34
Koordersiodendron pinnatum Merr.	0.70	0.52	0.29	0.83	0.64	0.41	1.69	1.17	0.74
Mangifera monandra Merr.	0.72	0.52	0.29	0.83	0.57	0.39	1.21	0.92	0.63
Semecarpus cuneiformis Blanco	0.52	0.29	0.25	0.56	0.43	0.29	1.12	0.79	0.54
18. Sapindaceae									
Guioa Perrottetii Bl.	0.45	0.38	0.32	0.66	0.43	0.25	2.00	1.48	0.96
Sapindus Saponaria L. var. *Turczaninowii* Vidal	0.41	0.25	0.14	0.50	0.33	0.19	1.60	1.20	0.68
19. Aceraceae									
Acer rubrum L.	0.64	0.49	0.27	0.61	0.49	0.32	1.24	0.84	0.50
IX. Malvales									
20. Tiliaceae									
Columbia serratifolia DC.	0.57	0.43	0.30	0.57	0.45	0.37	1.72	1.34	1.04
Grewia multiflora Juss.	0.34	0.25	0.14	0.37	0.25	0.16	1.09	0.75	0.48
21. Malvaceae									
Thespesia populnea (L) Soland. ex Corr.	0.32	0.25	0.14	0.28	0.25	0.21	1.45	1.09	0.36

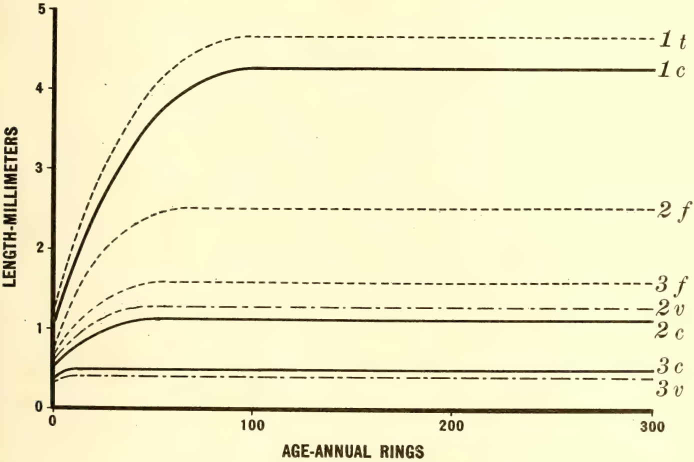
TABLE I (*Concluded*)

	Vessel-segments			Cambial Initials			Fiber Tracheids		
	Max.	**Mean**	Min.	Max.	**Mean**	Min.	Max.	**Mean**	Min.
22. Bombacaceae									
Bombycidendron Vidalianum Merr. and Rolfe	0.43	0.35	0.28	0.43	0.36	0.32	2.00	1.62	1.04
23. Sterculiaceae									
Heritiera littoralis Dryand.	0.37	0.31	0.25	0.36	0.30	0.27	1.88	1.42	0.96
Kleinhovia hospita L.	0.50	0.33	0.19	0.48	0.36	0.27	1.24	0.89	0.52
Pterospermum niveum Vid.	0.50	0.37	0.19	0.43	0.37	0.30	1.90	1.48	0.77
Sterculia foetida L.	0.48	0.35	0.30	0.45	0.37	0.32	2.72	2.11	1.64
Tarrietia sylvatica Merr.	0.34	0.27	0.18	0.34	0.28	0.21	1.96	1.57	1.12
X. PARIETALES									
24. Guttiferae									
Calophyllum Blancoi Pl. and Tr.	0.99	0.61	0.36	0.90	0.59	0.41	1.35	0.96	0.54
Garcinia dulcis Kurz.	1.24	0.80	0.48	1.09	0.80	0.52	2.88	2.01	1.12
Garcinia sp. (probably *lateriflora* Bl.)	1.04	0.78	0.48	1.02	0.74	0.52	2.52	1.88	1.28
25. Dipterocarpaceae									
Anisoptera thurifera Bl.	0.66	0.48	0.36	0.72	0.54	0.41	2.12	1.68	1.16
Vatica Mangachapoi Blanco	0.79	0.58	0.36	0.81	0.61	0.41	1.63	1.15	0.61
XI. MYRTIFLORAE									
26. Lythraceae									
Lagerstroemia speciosa (L.) Pers.	0.43	0.30	0.18	0.50	0.33	0.21	1.52	1.08	0.64
27. Lecythidaceae									
Barringtonia racemosa (L.) Roxb.	0.97	0.68	0.39	0.90	0.72	0.50	3.84	2.51	1.20
28. Rhizophoraceae									
Bruguiera parviflora W. and A.	1.20	0.94	0.60	1.28	0.99	0.64	1.88	1.32	0.96
Rhizophora sp. (probably *Candelaria* DC.)	0.95	0.59	0.46	0.95	0.73	0.30	2.12	1.56	1.16
29. Nyssaceae									
Nyssa sylvatica Marsh.	1.72	1.25	0.88	1.27	0.83	0.54	2.52	1.76	1.16
XII. UMBELLIFLORAE									
30. Araliaceae									
Shefflera odorata Merr. and Rolfe	1.00	0.82	0.56	0.97	0.84	0.66	0.76	0.52	0.37
B. METACHLAMYDEAE									
XIII. CONTORTAE									
31. Oleaceae									
Fraxinus americana L.	0.48	0.31	0.18	0.37	0.29	0.18	1.38	0.96	0.54
XIV. RUBIALES									
32. Rubiaceae									
Ixora philippinensis Merr.	1.13	0.62	0.50	1.17	0.76	0.43	1.78	1.18	0.66
Psychotria luzoniensis F. Vill.	0.95	0.67	0.37	1.08	0.70	0.45	1.53	1.12	0.61

VARIATIONS IN CROSS-SECTIONAL AREA AND VOLUME

The variations in the length of cambial initials are not neutralized by concomitant changes in the radial and tangential diameters of the cells. On the contrary, the cross-sectional area of the elongated meristematic cells tends to be somewhat larger in old than in very young stems, and in most gymnosperms than in dicotyledons. In other words, the basic fluctuations in *length* are paralleled by similar variations in *volume*.

The tracheary elements of the secondary xylem tend to increase in volume during differentiation. In the case of the tracheids of Coniferae this increase is due primarily to "radial" expansion and secondarily to elongation. The tangential diameter of the developing tracheids remains nearly constant. In arborescent and fruticose dicotyledons, on the other hand, the volume of fiber tracheids tends to be much influenced by elongation, and that of the vessel-segments by "tangential" as well as by "radial" expansion. As indicated by Sanio (1872) for *Pinus sylvestris* L., by Hartig and Weber (1888) for *Fagus sylvatica* L., and by Prichard and Bailey (1916) for *Carya ovata* (Mill.) K. Koch, the cross-sectional area and volume of tracheary cells tend to be larger in the outer than in the innermost growth layers of the stem. In gymnosperms, the changes in the volume of the tracheids in succeeding annual rings are closely dependent upon variations in the length and volume of the cambial initials, whereas, in many of the more highly specialized dicotyledons, the fluctuations in volume of the fiber tracheids and vessel-segments in various parts of the stem are due largely to changes which occur during the differentiation of the tracheary elements. In the dicotyledons as a group, the shortening of the cambial



TEXT FIG. 3. Normal length-on-age curves for cambial initials and tracheary cells in (1) typical conifer, (2) dicotyledon having primitive vessels, and (3) dicotyl having highly specialized vessels. *c*, cambium; *t*, tracheids; *f*, fiber tracheids; *v*, vessel-segments.

initials and fiber tracheids—which is closely correlated with the development and specialization of vessels—results in a reduction in volume of these elements, but the decrease in length of the vessel-segments frequently is more than compensated for by an increase in their cross-sectional area. Thus, there is less contrast between the volume of the tracheids in gymnosperms and that of the vessel-segments in dicotyledons than there is between the size of the cambial initials in the two groups of plants.

Significance of Size Variations in Cambium and Xylem

These fundamental types of cell size variations, and concomitant fluctuations in form and structure, are significant in the investigation of a number of cytological, morphological, and physiological problems, as well as in the study of the identification and mechanical properties of timber, and will be discussed in greater detail in subsequent papers.

In view of the numerous factors or complexes of factors which affect the dimensions and volume of cells, it is not surprising that contradictory conclusions have been reached by different investigators who have attempted to generalize concerning cell size after limited induction. The data at hand indicate very clearly that the undifferentiated, actively dividing and growing cells of the lateral meristem or cambium may vary greatly in size in certain plants and remain relatively constant in others. Therefore, very different conclusions concerning the constancy of cell size or of cell number may be expected from intensive experimental investigations, depending upon the particular plant or portion of a plant which is selected for study. Similar discrepancies may be expected concerning body size and cell size. Depauperate plants (physiological dwarfs) frequently have smaller tracheary cells and cambial initials than individuals of normal stature, indicating a close correlation between cell size and body size. On the other hand, a large dicotyledon may be composed of much smaller cells than a small conifer or dicotyledon of similar age, suggesting that variations in cell size are independent of fluctuations in body size.

Sachs (1892, 1893, 1895) and Strasburger (1893) almost simultaneously called attention to the fact that undifferentiated, actively dividing and growing cells of plants, such as occur in embryonic and meristematic tissues, are relatively minute, and concluded that this is undoubtedly due to the fact that the working sphere of the nucleus is very restricted. Strasburger found that in terminal meristems the ratio between the average diameter of the nuclei and of the cells is as 0.003–0.16 mm: 0.005–0.24 mm., or 2 : 3, and Sachs pointed out that, although plants vary enormously in their linear dimensions (0.001 mm. to 100 m.), the size of their constituent cells is relatively constant (0.001 to 0.05 mm.). Winkler (1916) reaches similar conclusions. He states that in meristematic somatic tissues the cells are of nearly uniform size and contain the diploid number of chromosomes, whereas in nonmeristematic somatic tissues, in which multinucleate protoplasts, nuclear fusions, and changes from the diploid to the tetraploid or polyploid condition are of frequent occurrence, many cells depart widely from the inherited, specific cell size of the plant. Therefore he suggests that there is a close correlation between cell size and chromosomal mass in both meristematic and non-meristematic somatic tissues.

Reconnaissance surveys of the higher plants indicate that the cambium should provide a favorable medium for testing the validity of these and similar generalizations concerning cell size, the working sphere of the nucleus, and the nucleo-cytoplasmic relation. Not only does the average size of the cambial initials fluctuate greatly in different groups of the Siphonogama, in different parts of a given individual, and in plants grown under different environmental conditions, but adjacent elements of the lateral meristem vary considerably in length, cross-sectional area, and volume. The cambial initials are of two distinct shapes and sizes: (1) numerous large, elongated cells, whose size variations have been described on preceding pages, and (2) scattered aggregations of small, more or less isodiametric elements which divide to form the horizontal sheets of radially disposed parenchyma, so-called medullary rays. The bulk of the divisions in both types of initials is periclinal, or parallel to tangents to the circumference of the stem or root. In other words, the large cells divide in a tangential, longitudinal plane which is a division plane of *maximal* area, whereas the ray initials form partition membranes that commonly are surfaces of *minimal* area. In gymnosperms and less highly differentiated dicotyledons, the cambium does not increase its periphery by radial, longitudinal divisions of the elongated initials and lateral enlargement of the products of such divisions. Instead, the cells elongate, sliding by one another, until they have attained a certain length. They then divide, by means of a pseudo-transverse partition, into two short halves which in turn elongate and divide.[8] Owing to the fact that the initials do not elongate and divide (transversely) in unison, there is usually a very considerable variability in the length and *pari passu* in the volume of adjacent fusiform elements. However, the volume of the more or less isodiametric ray initials is very much less than that of even the smallest fusiform initials, and is of the same general order of magnitude as that of the undifferentiated cells of the embryo or terminal meristem. Therefore, in any particular portion of the cambium of these plants it is possible not only to study cell division and the nucleo-cytoplasmic relation in adjacent fusiform initials of very different lengths and volumes, but to contrast them with similar phenomena in adjoining ray initials, which resemble the cells of the terminal meristem in size and shape. Furthermore, by proper experimental methods, the fusiform initials may be induced to divide into small isodiametric units of the general order of magnitude of the ray initials or embryonic cells, and subsequently to regenerate elongated elements of normal dimensions.

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[8] During this process of elongation, between successive transverse divisions, the cells continue to divide in the tangential, longitudinal plane.

A number of interesting cytological problems suggest themselves in this connection. (1) Are the large, elongated initials multinucleate or hyperchromatic in conformity with the generalizations of Sachs, Strasburger, Winkler, and others? (2) Do the nuclei divide mitotically or amitotically? (3) What is the nature of cytokinesis in cells which are several hundred times as long as they are wide, and yet divide longitudinally? These and similar questions will be considered in the next paper of this series.

SUMMARY

1. Reconnaissance surveys of the higher plants reveal striking variations in the dimensions and volume of the cells of the cambium and secondary xylem.

2. Certain of the size variations are purely somatic, whereas others are germinal.

3. In many plants the dimensions and volume of tracheary cells are determined primarily by those of the cambial initials, whereas in others they are due largely to changes which occur during the differentiation of the xylem.

4. These fundamental types of size variations, and concomitant fluctuations in form and structure, are significant in the investigation of various cytological, morphological, and physiological problems.

5. The cambium appears to be an unusually favorable medium for the study of problems relating to cell size and body size, the working sphere of the nucleus, the nucleo-cytoplasmic relation, and phenomena of cytokinesis in somatic tissues.

In conclusion, the writer wishes to express his indebtedness to Doctor E. D. Merrill, Director of the Philippine Bureau of Science, for his kindness in sending carefully preserved and identified specimens of a number of tropical plants.

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AN APPARATUS FOR DETERMINING SMALL AMOUNTS OF CARBON DIOXIDE

R. C. Wright

Certain investigations carried on by the Bureau of Markets in connection with the storage of fruits and vegetables require a simple and rapid method of determining small quantities of carbon dioxide in the air of both common and cold storage plants. The Orsat apparatus has been used to some extent in this work, but is open to objection because it will not measure small enough quantities, and the apparatus is somewhat heavy to carry about. Titration methods are, of course, the most accurate, but the necessary equipment, which is not easily portable, makes these methods practical only in what might be termed stationary experiments and where extreme accuracy is necessary.

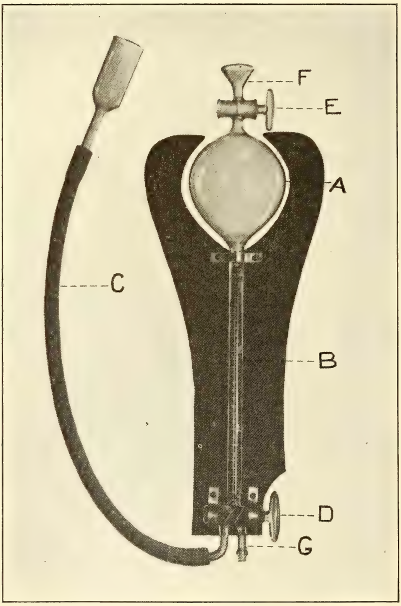

Fig. 1. See text for explanation.

The volumetric apparatus which has been developed by the writer has the advantages of being light, easily portable, measuring only 14 x 4 inches, and so simple in construction that it can be used by an unskilled operator. The apparatus is made entirely of glass and mounted on wood, and makes determinations ranging from 0.1 to 3.0 per cent. The calibrations, however, are sufficiently far apart so that readings by interpolation may be made to 0.05 percent. The apparatus can readily be used in the close, cramped quarters and poor light often found within storage rooms. Each determination takes from three to five minutes.

The carbon dioxide apparatus described herewith (see figure 1) consists of a bulb *A* and stem *B* of about 150 cc. capacity, a stopcock *E*, a balance tube *C*, a two-way stopcock *D*, and a funnel *F*. The apparatus is filled with air to be analyzed, and sodium hydroxide is introduced to absorb the carbon dioxide which is replaced by water entering from the balance tube *C*. The height of the column of water in the tube *B* gives directly in percentage the amount of carbon dioxide removed from the sample of air.

Following is a description of the method of operating the carbon dioxide apparatus. Wet the inside of bulb and stem *B*, then drain one minute. Fill the balance tube *C* with water. The water should rise in the balance tube just to fill the bore in stopcock *D*. Be sure no air bubbles are left inside the rubber tubing. Turn the stopcock *D* to make connection with the outlet *G*. Open the stopcock *E*, and by means of a bulb attached at *G*, pump into the apparatus sufficient air to get a representative sample, or place the mouth over *G* and draw through the apparatus sufficient air to get a good representative sample within. Turn *D* to connect with *C*. Lower the balance tube *C* till the level of water within is slightly below the bottom of tube *B*. Partially fill funnel *F* with a saturated solution of sodium hydroxide. Allow this to enter the apparatus slowly. Close *E* and raise the balance tube to allow two or three cubic centimeters of water to enter tube *B* along with the sodium hydroxide, then close *D* and tip the apparatus to allow the liquid to run into bulb *A*. Shake gently to allow the liquid to splash about in bulb *A* to facilitate absorption of carbon dioxide. Turn the apparatus upright. Open the stopcock *D* to connect with the balance tube *C*. Raise and lower the balance tube *C* as far as possible five or six times to force the rapid diffusion of sodium hydroxide, thus making the liquid in *C* of uniform density throughout. Allow liquid to drain down from the side of the apparatus for one minute, then hold the balance tube so that the top of the column of liquid within is on a level with that in tube *B*—thus correcting for atmospheric pressure. Read the height of liquid in tube *B*. (Because of the unequal capillarity due to the difference in diameters of tube *B* and the top of leveling tube *C*, when making a reading hold *C* so that the top edge of the meniscus is on a level with the bottom of the meniscus in *B*.) The reading gives directly in percentage the amount of carbon dioxide originally present. Rinse out after each determination.

When it is desired to analyze a sample of air from a container, such as a barrel or box, attach a bulb at *G* as usual, then connect the intake end of the bulb with a tube through which air from the container may be pumped, or attach a rubber tube at *G* through which air may be drawn from the container by placing the mouth over the funnel *F*, taking precautions to wash off all sodium hydroxide from about the sides of the funnel.

When operating the apparatus gloves should be worn and it should not be held close to the body, as the heat will expand the air within and true results will not be obtained.

Bureau of Markets,
U. S. Department of Agriculture

THE SECRETION OF INVERTASE BY PLANT ROOTS

Lewis Knudson

In an earlier paper (Knudson, 3) on the utilization of certain carbohydrates by green plants, the observation was repeatedly made that reducing sugars appeared in culture solutions containing sucrose. In discussing the possibility of invertase secretion the following statements were made:

> "It has not yet been definitely proved that the inversion of saccharose is due to invertase secreted into the culture solution. It is possible that the saccharose is inverted in the roots and the reducing sugars are secreted, but this is less probable. It is possible also that the enzyme may be released as a result of the death of root hairs or other cells of the root and that it is not secreted from living cells."

A few observations have been made by other investigators on this subject of enzyme secretion, but the observations have been only incidental to other investigations and the few reports are conflicting. It seemed desirable therefore to investigate thoroughly the possibility of enzyme secretion and particularly that of invertase, since it is this enzyme that is most likely to be found in the roots of plants. Accordingly the investigation here reported was undertaken. Not all the experiments performed are reported, but those omitted are in agreement with the results here given.

METHODS

The methods employed are essentially the same as those used by Knudson and Smith (4) in their experiments on the secretion of amylase. The plants were grown in water cultures under sterile conditions, that is, with the root system in a nutrient medium free of microorganisms and the seed removed from contact with the culture solutions. The type of culture is shown in figure 1. The details of manipulation are sufficiently described by Knudson and Smith (4) and need no repetition here.

Pfeffer's nutrient solution was used, with the substitution, however, of dibasic potassium phosphate for the monobasic potassium phosphate. The solution was prepared according to the following formula: $Ca(NO_3)_2$ 4 grams, K_2HPO_4 1 gram, KNO_3 1 gram, $MgSO_4 \cdot 7H_2O$ 1 gram, KCl 0.5 gram, $FeCl_3$ 50 milligrams, distilled water 6 liters. To this solution was added, when desired, sucrose.

In the use of Pfeffer's solution, according to the formula given, it is essential that precautions be taken to prevent acidification of the culture solution. When the nutrient solution is sterilized in an autoclave for a period of 30 minutes or more, there may result an acid solution. This appears to be due to the reaction between calcium nitrate and dibasic potassium phosphate, whereby there is produced some tri-calcium phosphate with the liberation of some nitric acid. If the sucrose is present, therefore, in the nutrient solution, a considerable portion may be inverted. The period of sterilization seems to be a factor in this acidification, for in some of the preliminary experiments no such inversion occurred when the entire nutrient solution was sterilized at one time.

Throughout the experiments here reported the following method was adopted. The nutrient solution was made up in two portions. Portion *A* includes all the salts except calcium nitrate. Portion *B* includes calcium nitrate only. To solution *A*, which is slightly alkaline, the sucrose was added. The solutions were originally made up double strength so that equal quantities of *A* and *B* would give the desired concentration of the sucrose and salts necessary for the nutrient solution. The method of procedure is as follows: When 1100 cc. of the culture solution is desired, 550 cc. of each solution (*A* and *B*) is accurately measured out. Solution *A* is placed in the culture flask and this is provided with a cotton stopper with a central tube. Through this tube is inserted the stem of a 9-centimeter funnel, and the funnel and neck of the flask are then covered with cotton to prevent any contamination after sterilization. Solution *B* is placed in a liter flask stoppered with cotton and the stopper and neck are also enclosed

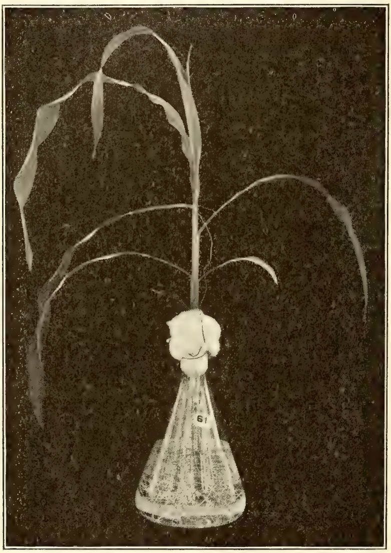

FIG. 1. See text for explanation.

with cotton. The two flasks are then sterilized in an autoclave for 30 minutes at a pressure of 15 pounds. When the solutions are cool, solution *B* is poured into the culture flask containing solution *A*. This transference of solution *B* to *A* takes place under conditions to minimize as much as possible the possibility of contamination. The funnel is then replaced by a cotton stopper and the cotton stopper and neck of the flask are covered again with cotton to prevent organisms and spores from lodging in the cotton stopper, circumstances which might cause contamination when the seedling is transferred to the culture flask. The flasks are permitted to stand several days before the seedlings are transferred, and at the time of transplanting any that show contamination are rejected.

Hydrogen-ion determinations were made by the indicator method, using mixtures of monobasic potassium phosphate and dibasic sodium phosphate prepared according to the methods of Soerensen (Prideaux, 7). These determinations were made at the outset of the experiment and also at its conclusion, and in some experiments mentioned subsequently the reaction of the culture solution was followed by adding to the culture solution the indicator, neutral red. The hydrogen-ion concentration is expressed as the logarithm (the base being 10) of the normality with respect to the hydrogen ions. The minus sign is understood; for example, P[H]7 refers to a hydrogen-ion concentration of 10^{-7} normal.

Sugar determinations in experiment 1 were made by Kendall's method (2), and the reducing sugar is expressed as invert sugar. In all the other experiments the volumetric method of Cole (1) was used. This method proved to be a rapid and accurate method for the purpose. The reducing sugars are expressed as glucose. In the use of both methods the solutions were standardized against prepared sugar solutions, the sugars used being of a very high degree of purity.

EXPERIMENTS

Experiment 1. In this experiment Canada field pea (*Pisum arvense* L.) was used. The culture vessels were pyrex flasks of one-liter capacity and the quantity of the nutrient solution was 1050 cc. Sterilization of the solutions was effected by autoclaving at 15 pounds pressure for 30 minutes. Seeds were sterilized by the use of calcium hypochlorite for one hour. The plants were grown in a greenhouse at an average temperature of 70° C.

At the conclusion of the experiment the culture solutions were tested for sterility by plating 1 cc. of each solution on an agar medium of Pfeffer's solution plus 1 percent sucrose. Only those cultures that proved to be sterile were analyzed. The results follow in table 1 and a typical culture is shown in figure 1.

There is in each of the culture solutions containing sucrose an appreciable gain in reducing sugars, but the gain is relatively slight as compared to the total amount of sucrose present. If the enzyme invertase is secreted, why

TABLE 1. *Canada field pea. Duration, Nov. 2 to Dec. 13, 1916: 42 days.*

Culture Solution	Culture Number	Water Transpired (Cubic Centimeters)	Dry Weight			Total Sugar in Culture Solution at End of Experiment, Calculated as Invert Sugar (Grams)	Sugar Absorbed by Plant, Calculated as Invert Sugar (Grams)	Reducing Sugar in Culture Solution at End of Experiment, Calculated as Invert Sugar (Grams)	Gain in Reducing Sugar, Calculated as Invert Sugar (Grams)
			Roots (Grams)	Tops (Grams)	Total (Grams)				
Pfeffer's + ½ per cent sucrose	1	210	0.130	0.375	0.505	4.584	0.216	0.541	0.233
" + ½ per cent "	2	230	0.170	0.368	0.538	4.544	0.256	0.600	0.292
" + ½ per cent "	3	170	0.082	0.215	0.297	4.652	0.148	0.497	0.189
" + ½ per cent "	4	120	0.140	0.140	0.280	4.572	0.228	0.491	0.183
" + ½ per cent "	5	150	0.100	0.280	0.380				

is there not a greater production of reducing sugars? The maximum increase in reducing sugar is only one fifteenth of the sucrose present.

Circumstances prevented at this time any incubation experiment with the culture solutions to determine whether or not there would result an increase in reducing sugars which might be taken as evidence of the presence of invertase.

Experiment 2. The culture methods and conditions were essentially like those of the preceding experiment. The nutrient solution was slightly modified by the substitution of ferrous chloride for ferric chloride, and the sucrose used was Merck's highest purity. The nutrient solution at the outset had a hydrogen-ion concentration of P[H] 6.80.

Two plants were used in the experiment: corn, variety Weber's Dent, and Canada field pea. Unfortunately most of the cultures of Canada field pea became contaminated, and data were obtained from only one sucrose culture.

An examination of table 2 reveals the fact that as usual a better growth is obtained with sugar than without. An exception is culture number 6, which was maintained in diffused light in the laboratory for ten days preceding the conclusion of the experiment.

In cultures 6 to 10 inclusive there was noted an increase in reducing sugars, but none was found in cultures 11 to 15 inclusive. The amount of reducing sugar in the sucrose cultures, while appreciable, is again relatively small compared to the total sugar present. In culture number 8 the unusually large amount of reducing sugar was undoubtedly due in part to contamination by a species of Penicillium which made its appearance during the last week of growth. The average hydrogen-ion concentration was at the conclusion of the experiment P[H] 7.35.

In order to determine whether or not the enzyme invertase is present in the culture solution, 500 cc. portions of the solutions were incubated for 14 days at a temperature of 32° C. As an antiseptic agent, 2 percent of

TABLE 2. *Corn. Duration, Dec. 31, 1918 to Feb. 10, 1919: 42 days.*

Culture Solution	Culture Number	Water Transpired (Cubic Centimeters)	Green Weight (Grams)	Dry Weight			Total Sugar at End of Experiment, Calculated as Sucrose (Grams)	Sugar Used, Calculated as Sucrose (Grams)	Reducing Sugar Present at End of Experiment (Grams)
				Roots (Grams)	Tops (Grams)	Total (Grams)			
Pfeffer's + sucrose . . .	6	300	21.5	0.220	0.970	1.19	3.769	0.984	0.583[1]
" + " . . .	7	326	27.0	0.410	1.420	1.83	4.314	0.439	0.434
" + " . . .	8	450	29.0	0.540	1.620	2.160	3.657	1.096	0.976[2]
" + " . . .	9	340	29.0	0.290	1.30	1.590	4.217	0.535	0.3125
" + " . . .	10	270	23.0	0.270	1.250	1.520	4.595	0.158	0.328
" + " . . .		Control solution no plant					4.753		trace
Pfeffer's.	11	300	13.2	0.120	0.610	0.730			No reducing sugar
"	12	310	16.5	0.230	1.050	1.280			
"	13	190	11.5	0.220	0.650	0.870			
"	14	168	10.5	0.130	0.520	0.650			
"	15	300	20.0	0.190	1.100	1.290			

[1] Kept in laboratory in diffused light for 10 days before analysis.

[2] Contaminated.

toluene was used. At the end of the 14 days, analyses were again made for reducing sugars. No increase was shown in any case after incubation except in number 3. In this case the amount of reducing sugar had nearly doubled, due undoubtedly to the enzyme invertase derived from the Penicillium contamination.

In the Canada field pea cultures only one of the sucrose cultures remained uncontaminated. The duration of growth in this case was 50 days, the green weight 14.95 grams, and the amount of reducing sugar present 0.448 gram. The total sugar present calculated as sucrose was 3.711 grams, and the amount of sugar as sucrose used was 1.042 grams. The non-sucrose cultures did not show any reducing sugars in the culture medium. As in the experiment with corn, 500 cc. of the culture solution was incubated for 14 days at a temperature of 32° C. No increase in reducing sugar was found at the end of that period.

Experiment 3. A white dent variety of corn was used and the plants were grown as before in the greenhouse. The duration of the experiment was from June 27 to July 29, a period of 32 days. The concentration of sucrose was ½ percent. In this experiment the hydrogen-ion concentration of the culture solution was again accurately determined by the indicator method, using anhydrous KH_2PO_4 and Na_2HPO_4 according to Soerensen and using neutral red as the indicator. In addition to determining the hydrogen-ion concentration, several cultures were provided, to each of which was added 1 cc. of a ½ percent solution of neutral red, the purpose being to follow the reaction during plant growth. This was possible for only about ten days, for the plant by the tenth day had absorbed all the indicator. From the outset the solution became increasingly alkaline, so that it was only at the outset that an acid reaction prevailed and then the hydrogen-ion concentration was only $10^{-6.7}$ normal.

The results of experiment 3 are similar to those of the preceding experiments. There is the usual increase in reducing sugars; the reaction of the solution at the outset was very slightly acid (P[H] 6.7), and at the conclusion slightly alkaline (P[H] 7.25 to 7.3)

Incubation experiments were made as in the previous experiments.

TABLE 3. *Corn. Duration of experiment, 32 days.*

Culture Number	Sugar as Sucrose at End (Grams)	Sucrose Used by Plant (Grams)	Reducing Sugar at End (Grams)	Gain in Reducing Sugar (Grams)	P[H] at Conclusion of Experiment
16.........	4.891	0.940	0.476	0.376	7.3
17.........	4.550	1.181	0.400	0.300	7.30
18.........	4.912	0.819	0.375	0.275	7.25
19.........	4.912	0.819	0.571	0.471	7.25
Control, no plant......	5.731		0.100		6.70

The duration of the incubation experiment was 14 days. Toluene 2 percent was added as the antiseptic agent, and the temperature of incubation was 35° C. No increase in reducing sugar over that found in the culture solution was noted after the period of incubation.

Experiment 4. This experiment was like the preceding. Canada field pea was used and the results appear in table 4.

TABLE 4. *Canada field pea. Duration, Sept. 4 to Sept. 22: 18 days.*

Culture Solution	Culture Number	Dry Weight			Reducing Sugar Present	Gain in Reducing Sugars
		Roots (Grams)	Tops (Grams)	Total (Grams)		
Pfeffer's solution + sucrose.......	1	0.221	0.254	0.475	839	0.333
" " + "	2	0.186	0.322	0.508	877	0.371
" " + "	3	0.223	0.277	0.500	820	0.314
" " + "	4	0.200	0.260	0.460	876	0.370
" " + " .... no plant					506	
Pfeffer's solution.................	1	0.113	0.216	0.339	No reducing sugars in these solutions	
" "	2	0.127	0.309	0.436		
" "	3	0.083	0.169	0.252		
" "	4	0.099	0.169	0.268		

Experiment 5. Culture in distilled water. In order to determine whether or not the character o the nutrient solution had any special significance with respect to the increase in reducing sugars, an experiment was made using in place of Pfeffer's solution merely distilled water to which was added 1 percent sucrose. Canada field pea was used and the duration of the experiment was 18 days. The conditions and methods of the experiment were similar to those of the preceding experiments.

Only one culture remained uncontaminated. The green weight of the plant was 1.35 grams, the reducing sugar in the culture solution (1000 cc.) at the conclusion of the experiment was 293 milligrams, while in the control there was only 138 milligrams; there was an increase therefore of 155 milligrams in the culture solution.

An incubation experiment was also made. 500 cc. samples were taken from the culture solution and from the control solution, and toluene was added. The solutions were incubated at 35° C. and then again analyzed for reducing sugars. The increase after nine days was but 5 milligrams.

Nutrient solution minus iron salts. Rice and Osugi (8) in working on the inverting power of various soils have presented evidence that inversion of sucrose may be affected by various colloids and suggest that the inversion may be due to adsorbed acids. In the Pfeffer's solution after sterilization there is precipitated ferric hydrate, and this precipitate is increased after a few days' growth of the plant. In order to determine whether or not the ferric hydrate might be responsible for the increase in reducing sugar, an experiment was performed in which iron was omitted from the culture solution. The methods were the same as for the previous experiments. Sucrose was supplied at a concentration of 0.5 percent. Corn was again used and the plants were grown for 18 days in the greenhouse. The dry weights of tops and roots were 0.725 grams and 0.200 grams respectively. Analyses showed 500 milligrams of reducing sugars, while the control solution had only 305 milligrams. The increase was therefore 95 milligrams.

Discussion

What is the cause of the increase in reducing sugar in the culture medium? Is the enzyme invertase excreted? The evidence is contrary to this idea. In no case was there obtained any increase in reducing sugar after incubation. It is possible, of course, that the enzyme invertase is excreted from the root in such small amounts that the reaction effected is very slight. It might be suggested, furthermore, that the culture solution is unfavorable to the invertase and that the latter is soon destroyed. It was noted, however, that whenever the culture solution became contaminated with a yeast or a fungus, there was a marked increase in reducing sugars, and that this increase continued after incubation. The incubation experiment for culture number 8 of experiment 2 yielded data in support of this statement. In accordance with the view of Rice and Osugi (8) it might be expected that the mucilaginous matter of the root and surrounding the root-cap cells as well as the cell walls might adsorb basic ions, the process resulting in a preponderance of hydrogen ions which might cause inversion of sucrose. But since the culture solution becomes increasingly alkaline in reaction with the advent of time, and since this alkalinity is due to the absorption of anions by the roots, it is reasonable to conclude that the zone about the roots is constantly of greater alkalinity than the "outer" regions of the culture solutions. In other words, the gradient of concentration of hydroxyl ions falls with increasing distance from the roots.

There is still another alternative. The cells of the root-cap are sloughed off, and it might be suggested that the root cells in dying yield reducing sugar to the culture solution. But, as stated in another paper (Knudson, 5), the writer has found that the root-cap cells that accumulate at the bottom of the culture flasks are not dead but apparently remain alive for a very considerable period. Examination of the sloughed off root-cap cells at the conclusion of the experiments revealed that they were alive and in good condition. Furthermore, the total weight of such cells would not be over 20 milligrams.

It seems to the writer that there is only one explanation to account for the accumulation of reducing sugars, and that is excretion of reducing sugars by the roots.

In accordance with this view, the procedure might be as follows: Sucrose is absorbed by the roots and inverted in the root cells by the enzyme invertase. Some of the sugar is used in growth, but there is a superabundance of reducing sugars and they accumulate in the root cells. At the outset there are practically no reducing sugars in the sucrose solutions. The concentration gradient between the reducing sugars in the cells and those outside is steep, and consequently some of the reducing sugars diffuse outward. With the progress of time the difference in concentration of reducing sugars becomes less, but probably it is considerable at all times, since at the conclusion of the experiment the concentration of the sucrose in the culture is much greater than that of the reducing sugars; and since there is a constant inward diffusion of sucrose, there results a constant production of reducing sugars in the plant cells.

In support of the view that the reducing sugars are excreted, the following experiment may be cited. Three corn plants which had grown for 30 days in Pfeffer's solution, each plant having a fresh weight of approximately 18 grams, were removed from the culture vessels and the roots washed in tap water. At 5 p.m. the plants were transferred to culture vessels so that their roots alone were bathed in a four percent solution of sucrose (Merck's highest purity). Three culture vessels were used and 400 cc. of the solution. The roots were kept in this solution for 16 hours. The plants were then removed and rinsed seven times in tap water, and then the plants were transferred to culture vessels this time containing distilled water. The plant roots remained in distilled water 7 hours. The total volume of distilled water was then reduced by evaporation to 100 cc. This was analyzed for reducing sugar and the determinations gave 14.5 milligrams of reducing sugar.

In another experiment plants were used which had been growing in a nutrient solution plus sucrose, and treated in the same way as in the preceding experiment. There were leached from the roots of four plants 75 milligrams of reducing sugar and 150 milligrams of sucrose.

The secretion of sugars by the roots of plants may seem at the outset to be a rather startling idea, yet theoretically there is no reason why this should not occur. Wächter (9) reported considerable excretion of sugars by slices of beets and onions when immersed in distilled water or in salt solutions, and recently much evidence has been presented showing the leaching of electrolytes from the roots of plants (see Merrill (6) for a review of literature).

SUMMARY AND CONCLUSIONS

1. Evidence is presented to show that Canada field pea (*Pisum arvense* L.) and corn (*Zea mays* L.) grown in the presence of sucrose cause an increase in reducing sugars in the culture solution.

2. The reaction of the culture solution is such as to be without influence on the sucrose.

3. Incubation experiments yielded negative results with respect to the presence of invertase.

4. The idea is held that the increase in reducing sugars is due to excretion of these from the roots.

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DAILY RHYTHMS OF ELONGATION AND CELL DIVISION IN CERTAIN ROOTS[1]

Ray C. Friesner

Introduction

The subject of periodicity of growth activities in plants is by no means a new one; in fact, it is one of the oldest. But a careful review of the available literature shows that there are still certain phases of the work which have not yet been thoroughly investigated. Two of these are embodied in the present paper, *viz.*, rhythms of elongation and rhythms of cell division in roots under constant environmental conditions.

Historical

Elongation

Aerial parts. Sachs (29,30) gives an historical account of the older literature up to his time. No attempt will be made to reproduce it here except to point out that the work before his time was all done on large plants which grew rapidly and which in most cases had to be observed in the open where external factors could not be controlled. Hence, the work was only of the grossest nature and led to no definite general conclusions. In 1872 Sachs (29) published the results of his study of the elongation of the stem in various plants, including *Dahlia variabilis*, Fritillaria, Polemonium, etc. In general he found that plants exposed to the alternation of darkness and light exhibited a single daily wave of elongation in which the maximum occurred shortly after sunrise, and the minimum shortly after sunset. This he formulated into his so-called "universal law." He further found that this daily periodicity is entirely absent from plants grown continually in the dark.

In 1873 Prantl (27) found, in studying the rate of growth in leaves, that curves for increase in width are very similar to those for increase in length, and that under normal conditions the maximum is reached in the morning from 6 to 9 and the minimum in the evening from 6 to 9. He found, further, that by changing the hours of illumination and darkness he could shift the times of maxima and minima at will, since for each change in the time of illumination and darkness there was a corresponding change in the times of maxima and minima. These results show clearly that the daily periodicity here is an induced one. In continuous darkness this periodicity was absent. In 1878 Stebler (33) published the results of similar observations on the growth of leaves of various species among which were *Secale cereale, Triticum vulgare, Allium Cepa,* Cucurbita, Melampsora, etc. His results seem to show that the time of maximum growth coincides with the time of maximum light intensity and that of the minimum growth with the time of minimum light intensity. Here, also, a single daily wave of elongation and increase in width was found, though its precise relation to the time of environmental changes was somewhat different.

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[1] Papers from the Department of Botany of the University of Michigan, no. 180.

In 1879 Baranetzky (3) published the results of his investigations on a number of species including *Gesneria tubiflora, G. cardinalis, Helianthus tuberosus* (plants from tubers), *H. annuus, Brassica rapa,* etc. In brief, he found that plants which exhibit a regular daily periodicity when exposed to the alternation of darkness and light gradually lose this periodicity when placed in continuous darkness. The time required for complete loss varies from two to three days in the case of *Gesneria tubiflora* to 14 days in that of *Helianthus tuberosus.* Further, the intensity of the rhythms decreases from day to day. Plants grown from the beginning in darkness exhibited no periodicity except in the case of the shoots of *Brassica rapa,* some of which showed a very clear and regular rhythm, others a poorly defined one, and still others showed none at all. He regards this as due to heredity. It could hardly be considered such according to the commonly accepted use of the word heredity. A better term would be the "persistence of the habit" in the tuber, and its subsequent transference to the shoot.

In 1892 Godlewski (10) published the results of his researches on the growth of epicotyls of *Phaseolus multiflorus.* In the experiments carried out in June 1888, he found that plants growing under normal conditions exhibited a single daily wave of elongation, the maximum coming in the afternoon and the minimum near midnight. The following year plants grown from seeds of the same collecting showed the waves to come somewhat later, the maximum at evening and the minimum in the morning. Further experiments with seeds of a different lot gave two daily waves. Plants exposed to uniform conditions showed a very considerable variation. In some no marked rhythms were found, and in others rather irregular and unsteady ones were found.

Underground parts. The earliest work on underground parts was that of Strehl (36) in 1874, on the radicle of *Lupinus albus* L. The conditions of his experiments were, however, far from normal, inasmuch as the seedlings were grown with their roots in water and kept near a west window where they were exposed to moderately strong light. In plants thus subjected to the alternation of day and night he found in most cases a single daily wave of elongation with maximum coming near midnight and minimum near noon. In a few cases two waves were found.

In 1891 MacMillan (23) reported the results of his experiments upon the potato tuber. He found that tubers growing in continuous darkness exhibited rhythmic pulsations in their growth, showing two, three, and four maxima and minima in the 24-hour period. He further found that the rhythms of the tuber were related to the periodicity of the aerial parts, but he thought it also probable that the tuber exhibited a rhythm of its own which was more or less obscured by the induced periodicity of the aerial parts. In 1901 Miss Gardner (9) reported the results of experiments on the growth of roots of *Pisum sativum* and *Vicia faba.* She found that roots exposed to the alternation of day and night elongated much more rapidly during the day than during the night. But the conditions of the experiment were far from normal, *viz.*, seedlings were placed in moist sawdust in wooden boxes with one glass face, and were made to grow in a horizontal direction.

A more recent work on elongation of underground parts is that of Kellicott (14) in 1904. In general, he found that curves for elongation of roots grown from bulbs of *Allium Cepa* exhibited three waves of elongation in the 24-hour period. Curves for different individuals were quite similar, though differing somewhat in the precise time of their maxima and minima. In general, the maxima came in the early morning and late afternoon and the minima came near noon and midnight. This work was done in the absence of any changes of environment, and hence is the first work definitely noting a regular rhythm not induced by external changes. A brief summary of the above account of investigations on elongation should note that (1) regular daily periodicity exists in the presence of regular daily changes in the environment; (2) this periodicity is gradually lost when the plants are exposed to constant conditions, though irregular and unsteady variations of the type called "autonomic" are reported; (3) the work of Kellicott on the root of Allium is the first to note any regularity in elongation of roots grown under constant conditions.

Cell Division

Lower plants. A great many statements are to be found in the older literature in regard to the time of day of nuclear and cell division among the Thallophytes. Thus Braun (4) notes that cell division in the formation of the gonidia of *Draparnaldia mutabilis* occurs between 6 A.M. and 11 A.M.; of *Stigeoclonium protensum*, between 6 A.M. and 10 A.M.; of *Cladophora tuberculata,* 8 A.M. to 2 P.M.; cell division in Spirogyra is most rapid during the night. Thuret (37) notes that the zoospores of Vaucheria are always liberated at about 8 A.M.; those of *Cutleria multifida* at daybreak; while those of *Enteromorpha clathrata* escape during the afternoon. Famintzin (8) corroborates Braun's statement in regard to Spirogyra. Strasburger (35) notes that cell division in Spirogyra is most rapid at 10–12 P.M., but may be delayed until the following morning if the plants are placed at a temperature of 0° to 5° C. during the night. De Wildeman (39), on the other hand, was unable to note any sensible difference, between day and night, in the rate of division in the cells of Spirogyra. His work was done during the winter months from material collected outside. Kurssanow (17) reports Zygnema as dividing most frequently between 9 P.M. and midnight. Numerous other examples from the older literature are cited by Karsten (12) which will not be reproduced here. Karsten (13) in the most recent paper has shown that the desmids: *Cosmarium Botrytis*, *Closterium moniliferum*, and *Mesotaenium Endlicherianum*, when grown under normal conditions of illumination, exhibit a regular daily periodicity in the rate of nuclear and cell division. Cosmarium exhibits three waves. The primary maximum (about 50 percent of all cells) occurs at 1 A.M., with secondary maxima at 5 and 11 A.M. The primary minimum (about 5 percent of all cells) occurs at 1–3 P.M., with secondary minima at 3 and 7 A.M. Similarly, Closterium and Mesotaenium exhibit regular waves in the percentage of cells undergoing division, differing only in detail from the condition above noted for Cosmarium. It should be borne in mind that all of the above cited cases are reported from experiments carried on under normal conditions of light and darkness.

Aerial parts of higher plants. The only published reports on periodicity of cell division in aerial parts known to the writer are those of Karsten (12 and 13). He used as material the apical meristem of seedlings of *Pisum sativum*, *Zea Mays*, and *Pinus austriaca*. Seedlings of Pisum grown in continuous darkness showed a very marked increase in the number of cells undergoing division between 9:30 P.M. and 2 A.M., with a minimum falling at 6 A.M., while the remainder of the day was occupied with smaller fluctuations. Similarly, the curve for *Zea Mays* grown in continuous darkness shows numerous minor oscillations during the day, with a very marked rising during the night until the crest is reached at about 4 A.M., from which time it falls back again to the day position. *This rhythm is independent of changes in illumination and temperature.* The effect of alternation of darkness and light was then studied. When plants were lighted during the day and darkened during the night, much the same sort of curve was obtained as when in continuous darkness. When the times of illumination and darkness were reversed, two waves appeared with maxima at 6 A.M. and 6 P.M. and minima at 4 P.M. and 10 P.M. When the plants were continually lighted the waves were much shorter and more numerous. Seedlings of *Pinus austriaca*, when grown under normal conditions, showed maxima at 4 A.M. and 4 P.M. and minima at 12 M. and 6 P.M.

Underground parts of higher plants. The earliest work of this sort done on roots is that of Lewis (21). In the preliminary notice of this work it is shown that roots from bulbs of *Allium Cepa*, when grown in water and under normal conditions of illumination, *i.e.*, regularly alternating day and night, show two waves in their rate of cell division. The maxima come at midnight and noon, and the minima at 4 A.M. and 4 P.M. When yellow light was used the maxima appeared as before, but with the minima at 8 A.M. and 8 P.M. In blue light the maxima occurred at 4 A.M. and noon, with the minima at 8 A.M. and 4 P.M. Finally, in continual darkness the maxima came at 4 P.M. and 8 A.M. with the minima at midnight and noon. Two waves were found in all these curves. The work of Kellicott (14) also shows two waves in the curves for cell division in roots of *Allium Cepa* grown from bulbs and in moist sawdust. The maxima came at 11 P.M. and 1 P.M. and the minima at 3 P.M. and 7 A.M. It should be noted that in his curve I no figures are given for 5 A.M., and that in his curve II the curve rises from the "normal" 11 P.M. maximum to a much higher one at 5 A.M. This point will be referred to again in connection with my own results. It should also be noted that a total difference of 13° C. appears between the highest and lowest temperatures, though there is apparently no direct relation to be noted in the curves between these temperature changes and changes in rate of cell division. Roots of *Podophyllum peltatum* also showed rhythms in their curve of cell division, though they were more numerous than in Allium.

Karsten (12) studied cell-division in the root tips of *Vicia faba* and *Zea Mays*. The curve for *Vicia faba* showed marked maxima at 9 A.M. and 9 P.M. with minima at 4 P.M. and 7 A.M., and a few minor variations. The curve for *Zea Mays* showed smaller oscillations throughout the entire 24-hour period, though the curve is higher from 5 A.M. to 6 P.M. and lower from 6 P.M. to 5 A.M., the highest point being reached at 7 A.M. and the lowest at 9 P.M. These experiments were conducted in continuous darkness.

Miscellaneous

It is of interest and indirect bearing on the present paper to mention a few other cases in which either rhythm or a daily periodicity is found. Pfeffer (26) found nearly the same results in regard to sleep movements of leaves, *viz.*, plants subjected either to constant illumination or to constant darkness lose their regular daily periodicity. In some cases autonomic waves are found under uniform conditions, and in others they are entirely absent. When present they show considerable variation both in different individuals and in different leaves of the same plant. Baranetzky (2) and Detmer (7) have shown that there is a single wave in the daily curve for root pressure. The maximum, while varying somewhat in different individuals, comes some time in the afternoon and the minimum about 12 hours later. In a recent paper, Romell (28) reports the same results from plants continually lighted: "Die Dauerlichtpflanzen, ohne Ausnahme, eine sehr ausgeprägte Tagesperiodicität in der Blütungskurve besässen." Humphreys (11) calls attention to the presence of two maxima and two minima in daily atmospheric pressure, and in electrical potential. Similarly, Dechevrens (6) reports, from observations in Jersey, the presence of a diurnal rhythm in electrical potential of the atmosphere. Kraus (15, 16) and Millardet (24) have shown that the daily periodicity of tissue tension is gradually lost when the plants are exposed to uniform conditions. Finally, Curtiss (5) has noted, under constant illumination, rhythms in the rate of transpiration of certain plants. A pronounced maximum occurs near midday, with other minor oscillations. He has further noted that the stomata are more responsive to stimuli in the morning than in the afternoon.

From the foregoing account of earlier work it is seen that in all cases when plants are exposed to the normal alternation of darkness and light a regular daily "periodicity" is thus induced; and that when these conditions are rendered uniform this "periodicity" is gradually lost. From the work of Kellicott (14), Karsten (12), and from the results of the present paper, it is seen that there is present, under uniform conditions, a "rhythm" which is entirely independent of the "periodicity" induced by environmental changes. This rhythm is concealed by the more prominent periodicity under normal conditions. Previous workers, including both Kellicott and Karsten, have failed to point out this difference. It is the object of this work to determine to what extent these rhythms are present in other species than those mentioned above, their probable cause, and their relation to the time of day.

MATERIALS AND METHODS

Materials

For the present study the following materials were used: radicles from seedlings of *Cucurbita Pepo* L., *Lupinus albus* L., *Pisum sativum* L., *Vicia faba* L., *Allium Cepa* L., and *Zea everta* Sturt.; and roots from germinating bulbs of *Allium Cepa* L., *A. canadense* L., and *A. cernuum* Roth.

Methods

Elongation. Seeds or bulbs were germinated in moist sawdust loosely packed in glass germinating chambers. These chambers had one face ground plane and polished, and measured 75 x 100 x 400 mm. The plane face was ruled in horizontal lines 2 mm. apart. Germination, except in a few cases, was secured at temperatures constant to within one degree C., though the temperatures used in different series ranged from 22° to 26° C. The cultural chambers were kept tilted a few degrees from the vertical while in the incubators, in order to have the root tips always growing directly along the inside of the chamber face. When observations were to be made, the chambers were taken from the incubators and placed before a horizontal microscope fitted with an eye-piece micrometer. The exact position of the tip of the growing root was then determined by measuring the number of micrometer spaces between it and the horizontal lines (on the face of the chamber) below and above it. In this way the exact position of the tip of the root was determined every hour throughout the course of the experiment, and the increments of growth calculated from the changes in this position. Since one eye-piece (micrometer) division was equal to 0.04 mm. absolute measurement, the growth increments could be measured accurately to 0.01 mm.

Cell Division. Root tips of the species to be studied were cut from seedlings (or germinating bulbs) germinated at 22° to 26° C. (but always constant to within one degree for any particular series) in moist sawdust in ordinary 4-inch pots. These tips were cut at intervals of two hours, 72 to 96 hours after the seeds or bulbs had been placed in the germinating pots. The tips were fixed 24 to 36 hours in medium chrom-acetic solution, washed, dehydrated, imbedded in paraffin in the usual way, cut into sections 10 microns in thickness, and stained in Delafield's haematoxylin. Only those slides showing sections cut exactly parallel to the long axis of the tip were used. Two or three slides were chosen for each hour, and from each slide chosen the median section and one on either side were marked off. The slides, having been previously labeled with a writing diamond, were now given a new number without regard to the first one, and all counting of dividing cells was done by this last number. The two numbers were not compared until the entire series had been counted, so that any influence due to a knowledge of the time of day of the particular slide being counted was avoided.

A typical observation. The slides and particular sections having been chosen for observation, the diameter of the section was then measured at a point where the root had attained a uniform diameter. Measurements were made by the eye-piece micrometer scale and are given accurate to the nearest 0.0085 mm. The diameter measured, the slide was moved by the mechanical stage to a point at a distance from the growing point of the tip equal to twice the diameter (measured where the root had attained uniform diameter) of the tip. The number of dividing cells in this area between the growing point and the imaginary line drawn across the section was then carefully counted. In order to facilitate the counting, a small rectangle was made by gluing four straight bristles (one for each side of the rectangle) into the eye-piece of the microscope. The section was then moved back and forth through this rectangle for counting. The number of cells dividing were recorded under the four phases: prophase, metaphase, anaphase, and telophase. All cells with nuclei between an evident spirem and the completion of the cell plate in the telophase were considered to be dividing. The area of the field observed was then determined by carefully counting the number of squares of a net eye-piece micrometer necessary to cover the field. This area was reduced to absolute measurement in square millimeters. It was soon discovered that the value so obtained very nearly approximated the value $7d^2/4$, where d equals the diameter of the section in millimeters. The amount of difference between the two methods mentioned above was always very small and constant for a given species. All calculations of areas given below were made from the latter formula.

Since it has been shown by a number of investigators, among whom are Amelung (1), Sanio (31), and, more recently, La Rue and Bartlett (18), that in corresponding organs of plants of the same species variation in cell size is so slight that variations in size of the part are due almost entirely to differences in cell number, and not in cell size, the number of dividing cells in all cases was reduced to the proper proportion for a common constant area of one square millimeter. This thus avoided error due to observation of roots of different sizes. This care was taken by Kellicott (14), but was omitted by Karsten (12 and 13). In all cases the area observed contained practically all of the dividing cells.

INVESTIGATION

Elongation

Pisum sativum. Seeds of two varieties, *viz.*, wrinkled (*gradus*), and smooth (No. 1 White Field of D. M. Ferry & Co.), were allowed to germinate, and when the radicles had attained a length of 50–70 mm. observations began. All observations were made in a dark room and at constant temperatures, so that the results obtained could not have been influenced by environmental changes of temperature and illumination. (In all the following plant and curve numbers it has been thought best to reproduce here the numbers as they actually occur in the original data). Space will permit the reproduction of but few of the mass of figures and curves upon which these results are based. Table 1 shows a representative set of elongation measurements; while in table 2 the times of maxima and minima of ten plants out of a total of 50 of this species studied are grouped. The other 40 are duplicates of one or other of those given in this table.

A study of curves 193 and 194 (figures in table 1) shows that elongation is rhythmic or oscillatory in nature, three waves of elongation occurring in the 24-hour period. Elongation is least rapid at 1–3 P.M., rises to a maximum at 5–7 P.M., with other maxima at 11 P.M. to 1 A.M., and 5–7 A.M., and minima at 9 P.M. and 3–5 A.M. These plants were of the smooth-seeded variety. Curve 174 again shows three waves of elongation; here, however, the maxima occur at 11 A.M., 9 P.M., and 5 A.M., and the minima at 7 P.M., 1 A.M., and 7 A.M. Curve 160 also shows three waves, with maxima at 1 P.M., 11 P.M., and 5 A.M., and minima at 11 A.M., 9 P.M., and 3 A.M. Comparison of these curves seems to show little uniformity. They are, however, not comparable for two reasons: (1) the first two are obtained from plants of the smooth-seeded variety and the latter two are from those of the wrinkled-seeded variety; (2) germination[2] in the case of the first two was begun at 9 A.M., in no. 174 it was begun at 8 P.M., and in no. 160 at 6 P.M. In order to make no. 174 comparable, with respect to time after initiation of activity, to plants started at 9 A.M., it will be necessary to move the entire curve (no. 174) backward 11 hours or forward 13 hours; similarly, no. 160 will have to be moved backward 9

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[2] In all cases throughout this paper the time stated for beginning of germination is the time when seeds were placed in the germinating chambers.

Table 1. *Pisum sativum. Elongation of Plants 193 and 194* (*Smooth-seeded variety*)

Time	Temp.	193		194	
10 A.M.	20.3	0.912		1.135	
11	20.8	0.955	1.867	1.045	2.180
12 M.	20.8	0.855		0.900	
1 P.M.	20.6	0.706	1.561	0.855	1.755
2	21.0	0.784		0.855	
3	21.0	1.116	1.900	0.765	1.620
4	21.0	1.180		1.045	
5	21.0	0.837	2.017	0.977	2.022
6	21.0	0.720		1.180	
7	21.2	0.675	1.395	1.135	2.315
8	21.0	0.315		0.900	
9	21.0	0.225	0.540	1.000	1.900
10	21.2	0.651		1.085	
11	21.0	0.457	1.108	0.865	1.953
12 N.	21.0	0.708		0.955	
1 A.M.	21.0	0.425	1.133	0.888	1.843
2	—	0.475		0.850	
3	21.0	0.475	0.950	0.850	1.700
4	21.0	0.750		0.600	
5	21.0	0.884	1.634	0.791	1.391
6	—	0.675		0.972	
7	21.0	0.675	1.350	0.972	1.944
8	—	0.585		0.522	
9	21.0	0.585	1.170	0.522	1.044

hours or forward 15 hours. The justification for this will be discussed later in connection with curves for cell division. The times of maxima and minima for plants given in table 2 are rewritten there on the basis of having started at 9 A.M. The lack of uniformity at first apparent disappears when

Table 2. *Pisum sativum. Grouping of Maxima and Minima of Elongation, Wrinkled-seeded Variety*

Plant	Maxima			Minima		
155	12 M.	10 P.M.	4 A.M.	10 A.M.	8 P.M.	2 A.M.
159	2 P.M.	10 P.M.	6 A.M.	10 A.M.	6 P.M.	4 A.M.
160	2 P.M.	8 P.M.	4 A.M.	12 M.	6 P.M.	2 A.M.
165	2 P.M.	8 P.M.	4 A.M.	10 A.M.	6 P.M.	2 A.M.
169	4 P.M.	8 P.M.	6 A.M.	12 M.	6 P.M.	4 A.M.
170	2 P.M.	10 P.M.	4 A.M.	12 M.	8 P.M.	6 A.M.
174	6 P.M.	12 N.	10 A.M.	2 P.M.	8 P.M.	8 A.M.
175	10 P.M.	12 N.	10 A.M.		4 P.M.	2 A.M.
	2 P.M.	12 N.	6 A.M.	8 A.M.	8 P.M.	12 N.

Summary

Maxima . . . . . . 2–6 P.M. 8–12 P.M. 4–6 (10)[3] A.M.
Minima . . . . . . 10 A.M.–2 P.M. 6–8 P.M. 2–4 (6)[3] A.M.

Smooth-seeded Variety

Plant	Maxima			Minima		
193	5 P.M.	1 A.M.	5 A.M.	1 P.M.	9 P.M.	3 A.M.
194	7 P.M.	11 P.M.	7 A.M.	3 P.M.	9 P.M.	5 A.M.

[3] Parentheses indicate an occasional variation in time to that enclosed by them.

the curves are plotted on an equal basis with respect to time after initiation of activity. Thus in general, in table 2, maxima occur at 2–6 P.M., 8–12 P.M., and 4–6 (10) A.M. in the wrinkled-seeded variety; and at 5–7 P.M., 11 P.M.–1 A.M., and 5–7 A.M. in the smooth-seeded variety; while the minima occur at 10 A.M.–2 P.M., 6–8 P.M., and 2–4 (6) A.M.; and 1–3 P.M., 9 P.M., and 3–5 A.M. respectively. It will be seen that the general character of the curves is the same for both wrinkled-seeded and smooth-seeded varieties. Both exhibit three waves of elongation in the 24-hour period, though the precise time of maxima and minima is usually slightly later in the smooth-seeded than in the wrinkled-seeded variety.

Except in a few cases, observations ceased at the close of the 24-hour period. In those few cases in which observations continued longer there was no material difference between the two days. The curve continued in the same oscillatory or rhythmic manner. The outstanding feature of these results is the rhythmic nature of elongation.

Lupinus albus. Seeds were germinated, and seedlings studied, in the same manner as above described for Pisum. In table 3 the maxima and minima of eight representative curves are grouped. A total of 23 different individuals was studied. It will be seen that here again three waves of elongation occur in the 24-hour period, with maxima at 1–3 P.M., 7 P.M.—1 A.M., and 5–7 A.M.; and minima at 11 A.M.—1 P.M., 5–9 P.M., and 3–5 A.M. Germination was begun at 9 A.M.

TABLE 3. *Lupinus albus. Grouping of Maxima and Minima of Elongation*

Plant	Maxima			Minima		
68...	3 P.M.	11 P.M.	7 A.M.	1 P.M.	9 P.M.	5 A.M.
69...	1 P.M.	1 A.M.	7 A.M.	11 A.M.	7 P.M.	5 A.M.
70...	3 P.M.	11 P.M.	7 A.M.	1 P.M.	9 P.M.	3 A.M.
71...	3 P.M.	7 P.M.	5 A.M.	1 P.M.	5 P.M.	3 A.M.
72...	1 P.M.	9 P.M.	7 A.M.	1 P.M.	7 P.M.	5 A.M.
73...	3 P.M.	11 P.M.	7 A.M.	1 P.M.	5 P.M.	3 A.M.

SUMMARY

Maxima......1–3 P.M. 7 P.M.–1 A.M. 5–7 A.M.
Minima......11 A.M.–1 P.M. 5–9 P.M. 3–5 A.M.

Curves 70 and 73 illustrate the character of elongation in two of these plants. While the corresponding waves (in regard to time of occurrence) in the various plants are not all of the same amplitude, the times of their maxima and minima are very close, and the character of the curves is very similar, indicating that once these activities are initiated they proceed in rhythmic fashion; and the time interval of the waves is a more or less nearly constant feature. The only earlier work on the root of Lupinus is that of Strehl (36). His results are not comparable with those of the present paper since his seedlings were exposed to the alternation of day and night, and hence any oscillations not induced by this alternation would be likely to be entirely concealed by the more prominent daily periodicity.

Allium Cepa. Roots from both germinating seeds and bulbs were used. The bulbs were uniform and of a medium-sized white variety, and the seed of the Yellow Danvers (D. M. Ferry & Co.) variety.

Roots from Bulbs. In table 4 are grouped the times of maxima and minima of the elongation of the roots of seven different plants. These are chosen to represent the various types of curves, and consequently show somewhat less approach to uniformity than when all curves are considered. Curves 272 and 296 show three waves of elongation in the 24-hour period. The maxima come at 7–9 A.M., 7 P.M., and 1 A.M.; and the minima at 1–3 P.M., 11 P.M., and 5 A.M. This type of curve is exhibited by about 75 percent of the plants. Comparison with Kellicott's (14) curves shows only slight differences in the exact time of occurrence of maxima and minima. A second type of behavior is illustrated in curves 261 and 264 where four waves are found in the 24-hour period. Three of these waves correspond closely, in regard to time, to those of the other plants which show three waves. A third type of curve is that shown by plant 263 where but two waves are found in the 24-hour period. Kellicott (14, page 545, fig. 7, curve II) shows a similar curve with but two waves. Two plants out of a total of 50 showed this type of curve.

TABLE 4. *Allium Cepa (bulb). Grouping of Maxima and Minima of Elongation*

Plant	Maxima	Minima
263....	11 A.M. 1 A.M.	9 P.M. 3 A.M.
261....	7 A.M. 1 P.M. 9 P.M. 1 A.M.	9 A.M. 5 P.M. 11 P.M. 5 A.M.
264....	11 A.M. 5 P.M. 9 P.M. 5 A.M.	3 P.M. 7 P.M. 11 P.M. 7 A.M.
254....	9 A.M. 9 P.M. 1 A.M.	3 P.M. 11 P.M. 7 A.M.
271....	11 A.M. 7 P.M. 3 A.M.	5 P.M. 11 P.M. 5 A.M.
272....	9 A.M. 7 P.M. 1 A.M.	3 P.M. 11 P.M. 5 A.M.
296....	7 A.M. 7 P.M. 1 A.M.	1 P.M. 11 P.M. 5 A.M.

SUMMARY

Maxima......	7–11 A.M.	7–9 P.M.	1–3 (5) A.M.
Minima......	1–5 P.M.	9–11 P.M.	3–7 A.M.

Roots from Seeds. Curves for elongation of roots from seedlings differ from those from bulbs mainly in that they are about equally divided between three- and four-wave types. In curves 275 and 288 three waves are shown, while curves 273, 274, and 276 exhibit four waves. All of these observations were made under identical conditions. Plants 275 and 276 grew beside each other in the same culture chamber, and a study of their curves shows how similar a four-wave curve is to one of three waves. It will be seen that the noon maximum comes two hours earlier in 276 than in 275, while the afternoon minimum comes two hours later in 276 than in 275. The other maxima, common to both, coincide; the difference in number of waves being due to the fact that 276 reaches its third maximum much earlier, sinks to a minimum, and then rises to a fourth maximum by the time 275

TABLE 5. *Allium Cepa (Seed). Grouping of Maxima and Minima of Elongation. Four-Wave Type*

Plant	Maxima				Minima			
276..	5 A.M.	11 A.M.	7 P.M.	1 A.M.	7 A.M.	5 P.M.	9 P.M.	3 A.M.
277..	9 A.M.	1 P.M.	7 P.M.	5 A.M.	11 A.M.	5 P.M.	1 P.M.	7 A.M.
273..	9 A.M.	1 P.M.	7 P.M.	1 A.M.	11 A.M.	5 P.M.	9 P.M.	3 A.M.
279..	9 A.M.	1 P.M.	7 P.M.	3 A.M.	11 A.M.	3 P.M.	9 P.M.	7 A.M.
283..	9 A.M.	1 P.M.	7 P.M.	3 A. .	11 A.M.	3 P.M.	9 P.M.	7 A.M.

SUMMARY

Maxima......5–9 A.M. (11 A.M.) 1–3 P.M. 7 P.M. 1–5 A.M.
Minima......(7) 11 A.M. 3–5 P.M. 9 P.M. (1 A.M.) 3–7 A.M.

has attained its third maximum. A similar comparison of curves 281 and 283 shows again how similar in general character are the curves of the two types. In curve 276 the extra wave appears in the hours just preceding and just following midnight, while in 283 the extra wave is only a very low-crested one and appears during the forenoon. In table 5 the maxima and minima of five different curves of the four wave type are grouped. It is seen that these curves are very similar and that there is very little overlapping of times of maxima and minima. In table 6 the summary of these curves is compared with five different curves of the three-wave type. It will thus be seen that the three-wave curves are, as individuals, very similar to those of the four-wave type, but differ among themselves primarily as to which of the waves (present in the four-wave curves) is omitted. The seeds for this work began germination at 9 A.M.

TABLE 6. *Allium Cepa (Seed). Comparison of Maxima and Minima of Elongation in Four-wave Curves with those of Three Waves*

	Maxima			
Four-wave type See table 5	5–9 A.M.	11 A.M.–3 P.M.	7 P.M.	1–5 A.M.
Three-wave type				
275	5 A.M.	1 P.M.	7 P.M.	
280		11 A.M.	5 P.M.	3 A.M.
286	5 A.M.	11 A.M.	7 P.M.	
288	7 A.M.		5 P.M.	11 P.M.
281	5 A.M.	9 A.M.	5 P.M.	

	Minima			
Four-wave type See table 5	(7) 11 A.M.	3–5 P.M.	9 P.M. (1 A.M.)	3–7 A.M.
Three-wave type				
275		3 P.M.	1 A.M.	7 A.M.
280		1 P.M.	9 P.M.	7 A.M.
286		5 P.M.	9 P.M.	7 A.M.
288	9 A.M.		7 P.M.	5 A.M.
281		3 P.M.	11 P.M.	7 A.M.

Cucurbita Pepo. Space will not permit so extensive a discussion as given above for Pisum, Lupinus, and Allium. Nothing unlike what we have already seen above was found in the study of this species. Curves

111 and 112, out of a total of 14 different plants studied, are given on Plate XXIV. In these, also, three waves of elongation occur in the 24-hour period.

Zea everta. For this study the White Rice (D. M. Ferry & Co.) variety was used. A single curve is shown on Plate XXIV for elongation. Too little work was done on this species to warrant definite conclusions. The curve, 102, shows two waves of elongation in the 24-hour period.

Summary for Elongation. Summarizing briefly in regard to elongation, we find that (1) elongation in all plants studied proceeds in a wave-like fashion, two to four waves being exhibited in the 24-hour period; (2) there is more or less variation among the various individuals of the same species in regard to the precise time of day of the occurrence of maxima and minima, though these can be arranged into definite groups which show very little overlapping of time (see tables 2–6); (3) it is indicated, though not definitely proven, in the case of Pisum, that the precise time of the occurrence of maxima and minima depends upon the time when germination was begun, and shows no relation to the actual time of day. This latter point will be taken up and definitely proven in connection with rhythms in cell division. This fact, if true, might also account for a great deal of the variation in elongation curves of plants of the same species placed in the germinating chambers at the same time, since it is possible that some of the seeds may have coats that are more permeable to water than others, and hence the precise time of initiation of metabolic activity would vary slightly.

Cell Division

Pisum sativum. For this work root tips from both the wrinkled-seeded and smooth-seeded varieties of peas were used. Curve 2 (figures in table 7) shows results obtained from a study of the wrinkled variety. Seeds were placed in germinating pots at 9 A.M. at a temperature of 25° C. and allowed to germinate for 72 hours. The radicles had attained a length of 20–50 mm. when killing and fixing began. It will be seen that three waves of cell division occur in the period of 24 hours. The three maxima come at 1 P.M., 5 P.M., and 5 A.M.; and the minima come at 11 A.M., 3 P.M., and 9 P.M. The two maxima coming at 5 P.M. and 5 A.M. are about equal in extent. It will be noticed throughout the curves that follow that those waves in the various curves from roots of the same variety of seed which are coordinate in regard to time of appearance, are not always of the same amplitude. Kellicott (14) found similar results in *Podophyllum peltatum.* A study of the figures from which this curve is drawn (table 7) shows remarkable uniformity of the different roots for the same hour. Only at 5 and 11 A.M. do any appreciable differences occur, and then they are of such a nature that they do not affect the character of the curve. Curve 27 shows results from a similar study of the smooth-seeded variety. These seeds

TABLE 7. *Pisum sativum. Wrinkled-seeded Variety. Figures for Cell Division, Curve 2. Germination began at 9 A.M. January 24–25, 1918*

Time	Temp.	Diam. Area	Dividing Cells				Total	Total × C[6]	Ave. 1 Tip	Ave. 3 Tips
			Pro.	Meta.	Ana.	Telo.				
9 A.M. . .	25.0	.748[4]	160	47	11	34	252	257		
		.977[5]	180	49	8	36	273	279	284	
			233	47	7	23	310	317		
		.935	262	63	8	35	368	240		
		1.529	239	52	13	44	348	227	241	259
			263	66	10	55	394	257		
		.748	190	45	8	25	268	274		
		.977	147	40	9	32	228	233	252	
			166	43	5	32	246	251		
11 A.M. . .	25.0	1.03	107	42	3	9	161	87		
		1.856	108	37	4	14	163	88	98	
			168	38	4	15	225	121		
		.858	112	50	12	46	220	168		
		1.188	83	25	7	35	150	115	138	139
			98	40	10	28	176	135		
		.901	188	61	6	33	288	203		
		1.421	132	55	6	30	223	157	180	
1 P.M. . .	26.0	.935	249	50	16	37	352	230		
		1.529	351	61	12	32	456	301	278	
			320	51	9	36	416	272		
		.867	327	39	5	29	400	304		
		1.315	320	43	11	38	412	313	319	341
			370	43	6	25	444	337		
		8.42	306	62	13	66	447	361		
		1.237	288	58	13	55	414	334	339	
			272	75	8	45	400	323		
3 P.M. . .	26.0	.875	221	34	4	29	288	214		
		1.340	288	36	12	22	358	265	240	
			239	42	17	27	325	242		267
		.859	292	51	14	28	385	295		
		1.290	307	54	9	28	398	305	294	
			285	44	10	32	371	284		
5 P.M. . .	25.5	.988	516	87	14	62	679	396		
		1.707	598	62	11	50	721	421	405	
			519	80	31	66	696	406		
		.918	507	46	10	56	619	419		
		1.475	412	59	9	41	521	352	367	397
			374	59	16	38	487	330		
		.825	368	63	10	46	487	408		
		1.189	368	67	18	33	486	408	419	
			414	54	12	44	524	440		

[4] Diameter of section in millimeters, always upper number.

[5] Area counted, see page 386.

[6] C = Constant necessary for reduction of figures to common area of 1 sq. mm.

TABLE 7 (*Continued*)

Time	Temp.	Diam. Area	Dividing Cells				Total	Total × C	Ave. 1 Tip	Ave. 3 Tips
			Pro.	Meta.	Ana.	Telo.				
7 P.M. . .	25.25	.867	375	68	15	64	522	397		
		1.315	367	83	11	55	516	392	403	
			387	88	19	60	554	421		
		.782	325	49	8	49	431	402		
		1.070	328	41	9	33	411	384	375	368
			278	43	8	33	362	338		
		.850	282	64	14	51	411	326		
		1.261	324	62	11	59	456	361	327	
			242	62	15	53	372	295		
9 P.M. . .	25.25	.833	156	52	11	34	253	208		
		1.212	182	44	7	40	273	225	226	
			191	55	12	40	298	246		
		.910	204	49	11	43	307	211		
		1.448	178	57	11	47	293	203	204	237
			170	54	14	47	285	197		
		.842	237	65	10	36	348	281		
		1.237	256	66	10	30	362	292	280	
			227	67	6	32	332	268		
11 P.M. . .	25.0	.884	301	56	9	30	396	289		
		1.368	309	43	21	35	408	298	284	
			277	49	13	25	364	266		
		.774	231	56	7	42	336	321		
		1.047	231	61	12	38	341	325	316	271
			200	63	17	38	318	303		
		.833	148	42	7	22	219	180		
		1.212	196	33	12	37	278	229	213	
			189	37	8	45	279	230		
1 A.M. . .	25.0	.816	255	55	12	40	362	311		
		1.165	326	64	11	38	349	299	315	
			273	65	10	45	393	337		
		.842	274	60	10	37	381	308		
		1.237	274	59	10	56	399	322	316	295
			260	70	7	57	394	318		
		.910	246	59	8	48	361	249		
		1.448	257	85	6	23	371	256	256	
			271	58	18	34	381	263		
3 A.M. . .	25.0	.979	382	53	5	15	455	272		
		1.677	322	37	12	24	395	237	265	
			376	57	8	30	472	276		
		.807	343	45	10	30	428	375		
		1.140	291	56	12	24	383	336	365	301
			331	61	20	28	440	385		
		.884	355	40	4	31	653	314		
		1.369	258	38	9	20	023	237	272	
			270	45	10	39	44	266		

TABLE 7 (*Concluded*)

Time	Temp.	Diam. Area	Dividing Cells				Total	Total × C.	Ave. 1 Tip	Ave. 3 3 Tips
			Pro.	Meta.	Ana.	Telo.				
5 A.M. . .	25.0	.988	445	53	15	36	549	319		
		1.707	319	52	12	47	430	251	279	
			345	66	19	30	460	268		
		.918	497	71	23	39	630	427		
		1.475	510	87	25	59	681	461	438	381
			455	89	23	66	633	428		
		.850	456	56	16	44	572	452		
		1.261	360	64	12	37	473	375	425	
			437	74	14	45	570	450		
7 A.M. . .	24.0	.808	275	50	11	52	388	340		
		1.140	311	60	12	38	421	369	341	
			258	53	13	33	357	313		
		.859	240	57	10	53	360	278		
		1.290	253	63	11	52	379	292	279	305
			238	65	2	40	345	266		
		.791	218	46	14	36	314	285		
		1.093	221	59	6	47	333	302	294	
			202	55	12	56	325	295		

were placed in germinating pots at 9 A.M. and incubated for 72 hours at a temperature of 22°–23° C. Here also it will be seen that three waves occur in the 24-hour period. The maxima come at 3 P.M., 9 P.M., and 1 A.M.; and the minima at 11 A.M., 7 P.M., and 11 P.M. A comparison of curves 2 and 27 shows that the first two maxima of curve 2 each come just eight hours earlier (or 16 hours later) than two of curve 27, while the third maximum departs somewhat from this time relation. A similar relation exists between the minima.

Let us now turn to evidence in support of the contention that the time of occurrence of maxima and minima is related to the time of initiation of activity and not to time of day. Curve 28 is the result obtained from root tips of the smooth-seeded variety grown at the same time and in the same incubator as those represented by curve 27, with the difference that the seeds for curve 28 were placed in the germinating pots at 2 P.M., instead of 9 A.M. of the same day. In curve 28 three marked maxima occur with a very small fourth. Omitting, for the present, this extra small wave, we find maxima occurring at 7 P.M., 3 A.M., and 7 A.M., and minima at 3 P.M., 11 P.M., and 5 A.M. Now it will be seen that these seeds were started to germinate just 5 hours later than those of curve 27. Since root tips were cut and fixed every two hours, a difference of precisely five hours would not appear in the curves as such, but rather as a four- or six-hour difference. Comparison of the two curves will show that the 7 P.M. maximum of curve 28 is just four hours later than the 3 P.M. maximum of curve 27; similarly, the 3 P.M. and 11 P.M. minima of curve 28 are just 4 hours later than the

11 A.M. and 7 P.M. minima of curve 27; while the 3 A.M. and 7 AM.. maxima, and the 5 A.M. minimum of curve 28 are each just 6 hours later than the corresponding maxima and minimum of curve 27. Thus the entire curve 27 is earlier than curve 28 by an amount of time equal to the difference in time between the beginnings of germination. As further evidence on this point, a third series of root tips were cut at the same time and under identical conditions. The seeds for this third series were placed in the germinating pots at 8 P.M. Curve 31 shows the results of this study. In curve 28 a fourth wave was merely indicated, while in curve 31 there are definitely and clearly four waves. It is seen that because of the difference between the times when seeds were placed in germinating pots there would be expected to be a difference of just eleven hours between the times of initiation of activity in curves 27 and 31, and six hours between curves 28 and 31. Table 8 shows the maxima and minima of these curves correlated in respect to time (after initiation of activity) of their occurrence.

Table 8. *Pisum sativum. Correlation of Maxima and Minima of Curves 27, 28, and 31*

27	28		31		
Germination Began at 9 A. M.	Germination at 2 P. M.	Diff. from 27; 5 Hrs.	Germination at 8 P. M.	Diff. from 27; 11 Hrs.	Diff. from 28; 6 Hrs.
Maxima					
3 P.M.	7 P.M.	4	3 A.M.	12	8
9 P.M.	3 A.M.	6	7 A.M.	10	4
1 A.M.	7 A.M.	6	3 P.M.	14	8
	11 A.M.				
Minima					
11 A.M.	3 P.M.	4	9 P.M.	10	6
7 P.M.	11 P.M.	4	5 A.M.	10	6
11 P.M.	5 A.M.	6	11 A.M.	12	6
	9 A.M.				
			1 A.M.		

A study of this table shows that the same relation exists between curves 28 and 31, and 27 and 31, as is shown above between curves 27 and 28, *viz.*, there are in both curves 28 and 31 waves corresponding, in time after initiation of activity, to each of the three waves shown in curve 27. The extra (fourth) waves appearing in curves 28 and 31 not only do not have a corresponding wave in curve 27, but also seem not to be correlative to each other.

A further experiment of this same nature was carried out in which two series of peas of the smooth-seeded variety were placed in germinating pots at 9 A.M. and incubated at 24–25° C. for 48 hours. They were then removed from the incubators to a refrigerator where a recording thermometer showed the temperature to vary between 6.0° and –0.5° C. for a period of 48 hours. During the time of refrigeration, control plants were kept growing in the glass culture chambers used for elongation studies, and their elongation was measured. The elongation figures (omitted for lack of space) show that the temperature was sufficiently low to inhibit all but the slightest activity. After the plants had been in the refrigerator for nine hours, and from that time until the end of the period of refrigeration, the amount of elongation of the individual plants ranged from 0.018 to 0.079 mm. per hour. In six hours after being taken from the refrigerator and incubated at a temperature of 24°–25° C. these same control plants had regained their normal rate of elongation for that temperature. At the end of the refrigeration period the seedlings from which root tips were to be cut were also incubated at a temperature of 24°–25° C. Series 33 (curve 33) was removed from the refrigerator at 9 A.M., and series 35 (curve 35) was removed at 1 P.M. A comparison of the two curves (table 9) shows that there are present, again, three waves, and that the times of two of the maxima and one of the minima are just four hours later in curve 35 than in curve 33, while the 7 P.M. minimum of curve 35 is six, instead of four, hours later than the 1 P.M. minimum of curve 33; and that the 11 P.M. minimum and 1 A.M. maximum of curve 35 are each but two hours later than the corresponding minimum and maximum of curve 33. Hence, in general, these curves also differ from each other by a time interval equal to the difference in time between their initiation of activity after refrigeration.

TABLE 9. *Pisum sativum. Correlation of Maxima and Minima of Curves 33, 35, and 27*

33	35		27		
Removed from Refrigerator at 9 A. M.	Removed from Refrigerator at 1 P. M.	Diff. from 33	Germination Began at 9 A. M.	Diff. from 33	Diff. from 35
Maxima					
5 P.M.	9 P.M.	4	3 P.M.	2	6
11 P.M.	1 A.M.	2	9 P.M.	2	4
5 A.M.	9 A.M.	4	1 A.M.	4	8
Minima					
1 P.M.	7 P.M.	6	11 A.M.	2	8
9 P.M.	11 P.M.	2	7 P.M.	2	4
1 A.M.	5 A.M.	4	11 P.M.	2	6

A comparison of curves 33 and 27 (table 9) shows that with but one exception the maxima and minima of curve 33 occur just two hours later than the corresponding waves of curve 27. This exception is found where the 5 A.M. maximum of curve 33 comes four, instead of two, hours later than the 1 A.M. maximum of curve 27. While the particular amount of difference in time between waves in curves 33 and 27 has no special significance, the fact that the time interval between waves of one curve is the same as that between waves of the other curve, taken together with the relation we have just seen existing between all these other curves of Pisum, proves that these rhythms are regular and definite and not mere chance variations. It further indicates the truth of the contention that the time of occurrence of maxima and minima is related to the time of initiation of activity, and not to actual time of day.

We note from this study of cell division in Pisum that (1) once activity is initiated it proceeds in a rhythmic fashion; (2) in general, three waves are shown in the 24-hour period; (3) the exact time of appearance of maxima and minima is dependent upon the time of initiation of activity and shows no relation to time of day.

Lupinus albus. Curves 1 and 13 show the results of a study of cell division in this species. These curves, again, show three waves. Curve 1 shows the first maximum and minimum coming about four hours earlier than the corresponding wave in curve 13, though the general character of the two curves is strikingly similar and their rhythmic nature is well demonstrated. It should be mentioned that the two curves were obtained from seeds of different lots. The seeds in both cases began germination at 9 A.M.

Allium Cepa, Roots from Bulbs. Curve 10 shows three waves of cell division with maxima coming at 1 P.M., 9 P.M., and 5 A.M.; and the minima at 3 P.M., 1 A.M., and 7 A.M. In comparing this curve with those given by Kellicott (14) it is found that the 1 P.M. maximum and the 3 P.M. and 7 A.M. minima correspond to maximum and minima at similar times in his curves; while the 9 P.M. maximum of curve 10 comes just two hours earlier than the 11 P.M. maximum of his curve I, and one hour later than the 8 P.M. maximum of his curve II (page 563 of his paper). The 1 A.M. minimum and 5 A.M. maximum of curve 10 find no equivalents in his curve I. In his curve III, however, a third maximum occurs at 5 A.M. It should be noted that no figures are given for 5 A.M. in his curve I, and hence it is possible that a third maximum may have been missed at this hour. Curve 24 is drawn from data obtained a year after that of curve 10, and from a different lot of bulbs. Other conditions were the same in both. In comparison it is seen that the noon maximum of curve 24 comes at 11 A.M. instead of 1 P.M.; the afternoon minimum comes at the same time as in curve 10; while an additional low-crested wave, with maximum at 5 P.M. and minimum at 7 P.M., appears between the times of the first and second waves of curve 10. The remaining waves are the same in both. Curve 24 thus shows four waves instead of the usual three. In comparing these curves with those of Kellicott's on Allium we note that the main difference is the larger number of waves here shown. Kellicott used much lower temperatures than those used in the present work, and it is possible that this may account for the smaller number of waves found in his curves.

Roots from Seeds. Curve 12 shows results from a study of roots from seeds of the Yellow Globe variety. It will be seen that there is little difference between this and curve 10 (from bulbs), three waves being found in each case. The essential difference is found in the fact that the curve does not drop so suddenly to a minimum after both the 1 P.M. and the 9 P.M. maxima, in curve 12, as does curve 10.

Zea everta. Curve 7 shows results obtained from a study of roots from seedlings of the White Rice variety. Germination began at 9 A.M. It will be seen that the curve is much more oscillatory in character. Karsten

(12) found much the same condition in *Zea Mays.* While the number of waves found in the 24-hour period is higher than in the case of any other species studied, yet the fact that mitotic activity proceeds in waves or rhythms is none the less clearly demonstrated.

Vicia faba. Curve 5 shows results obtained from a study of roots of *Vicia faba.* Germination began at 9 A.M. It will be seen that two waves of cell division occur in the 24-hour period. Maxima occur at 5 P.M. and 7 A.M. and minima at 1 P.M. and 1 A.M. Comparison of this curve with the figures given by Karsten (12, page 9) shows that he, too, found two extensive waves of cell division with maxima coming at 10 A.M. and 9 P.M., and minima at 4 P.M. and 7 A.M. Thus the maxima of curve 5 come just three and four hours earlier, and the minima three and six hours earlier, than in Karsten's results. Besides the two more extensive waves it will be seen that his figures show two very small waves, one coming in each larger wave. He, however, did not take into consideration variations in size of the sections counted, and this, taken together with a possible difference in time of beginning germination, probably accounts for the differences between his results and those of the present paper.

Allium cernuum. For this study bulbs were collected in the field in October, stored in boxes of soil, and kept in the open until ready for use the following January. Upon germination each bulb produced from two to four roots. Curve 23 shows results from this study; it will be seen that four very marked waves occur in the 24-hour period.

Allium canadense. For this study the small aerial bulblets were collected in October and stored in a dry, cool place until ready to be used the following January. Curve 22 shows results from this study. It will be seen that five waves of cell division occur in the 24-hour period.

A brief summary of the results obtained from this study of cell division shows the following facts: (1) the curve of cell division in all plants studied exhibits a number of oscillations in the 24-hour period, in the majority of plants three; (2) the exact time of occurrence of maxima and minima is dependent upon the time of initiation of activity and not on time of day.

RELATION BETWEEN ELONGATION AND CELL DIVISION

Historical

De Wildeman (39) has shown by exact measurements that cells of Spirogyra do not elongate during mitosis, while in the staminal hairs of Tradescantia there is very slight elongation of the cell during early prophases but none at all during the later stages. Ward (38) has shown in his study of cell division and elongation of filaments of *Bacillus ramosus* Fraenkel that elongation proceeds in a wave-like fashion and that "the period of cell division entails more or less cessation of growth." Kellicott (14) has shown that, in general, the same thing is true of elongation and cell division in roots from bulbs of *Allium Cepa*, *i.e.*, the times of maxima of cell division are near the times of minima of elongation and *vice versa*. It should be noted that the observations of de Wildeman (39) and Ward (38) were made directly upon the dividing cell while it *was* dividing. The two processes were observed in one and the same cell. Such direct observation in the case of root tips is, of course, out of the question.

Experimental

Pisum sativum. In table 10 the times of maxima and minima of elongation and cell division in Pisum are compared. It is seen that in both the wrinkled-seeded and smooth-seeded varieties the times of maxima of elongation correspond very closely to the times of minima of cell division, and *vice versa*. A single exception is found in each variety: in the wrinkled-seeded variety the 11 A.M. minimum of cell division comes considerably later than the corresponding maximum of elongation in the majority of plants; and in the smooth-seeded variety the 11 A.M. minimum of cell division comes much earlier than the corresponding maximum of elongation. With the exception of this one divergence in each case there is a very close reciprocal relation existing between the rapidity of elongation and the number of cells undergoing division.

TABLE 10. *Comparison of Maxima and Minima of Elongation and Cell Division in Pisum*

WRINKLED VARIETY

Elongation Maxima (see table 2)	2–6 P.M.	8–12 P.M.	4–6 (10) A.M.
Cell Division Minima (see curve 2)	3 P.M.	9 P.M.	11 A.M.
Elongation Minima	10 A.M.–2 P.M.	6–8 P.M.	2–4 (6) A.M.
Cell Division Maxima	1 P.M.	5 P.M.	5 A.M.
SMOOTH VARIETY			
Elongation Maxima (see table 2)	5–7 P.M.	11 P.M.–1 A.M.	5–7 A.M.
Cell Division Minima (see curve 27)	11 P.M.	11 P.M.	7 A.M.
Elongation Minima	1–3 P.M.	9 P.M.	3–5 A.M.
Cell Division Maxima	3 P.M.	9 P.M.	1 A.M.

TABLE 11. *Comparison of Maxima and Minima of Elongation and Cell Division in Lupinus*

Elongation Maxima (see table 3)	1–3 P.M.	7 P.M.–1 A.M.	5–7 A.M.
Cell Division Minima			
Curve 1	3 P.M.	1 A.M.	5 A.M.
Curve 13	7 P.M.	1 A.M.	7 A.M.
Elongation Minima	11 A.M.–1 P.M.	5–9 P.M.	3–5 A.M.
Cell Division Maxima			
Curve 1	9 A.M.	11 P.M.	3 A.M.
Curve 13	1 P.M.	11 P.M.	3 A.M.

Lupinus albus. In table 11 the maxima and minima of elongation and cell division in Lupinus are compared. It will be seen that here again there is a very close reciprocal relation existing between elongation and cell division. A single large divergence occurs in the case of the 7 P.M. minimum of cell division in curve 13.

Allium Cepa. In table 12 the maxima and minima of elongation and cell division in *Allium Cepa* are compared. In the case of roots from bulbs we find, again, very nearly a reciprocal relation between rapidity of elongation and number of cells undergoing division. Another divergence is seen in the case of the 3 P.M. minimum of cell division in both curves 10 and 24 (or 7–9 P.M. maximum of elongation).

TABLE 12. *Comparison of Maxima and Minima of Elongation and Cell-Division in Allium Cepa*

ROOTS FROM BULBS

Elongation Maxima (see table 4)	7–11 A.M.	7–9 P.M.	1–3 (5) A.M.
Cell Division Minima			
Curve 10	7 A.M.	3 P.M.	1 A.M.
Curve 24	7 A.M.	3 P.M.–7 P.M.	1 A.M.
Elongation Minima	1–5 P.M.	9–11 P.M.	3–7 A.M.
Cell Division Maxima			
Curve 10	1 P.M.	9 P.M.	5 A.M.
Curve 24	11 A.M.–5 P.M.	11 P.M.	5 A.M.

ROOTS FROM SEEDS

Elongation Maxima (see table 5)	5–9 A.M.–(11 A.M.)	1–3 P.M.	7 P.M. 1–5 A.M.
Cell Division Minima			
Curve 12	7 A.M.	5 P.M.	3 A.M.
Elongation Minima	11 A.M.	3–5 P.M.	9 P.M. 3–7 A.M.
Cell Division Maxima	1 P.M.		9 P.M. 5 A.M.

In the case of roots from seeds all of the maxima and minima of cell division find corresponding minima and maxima respectively in elongation so that the reciprocal relation here is quite evident except for the extra fourth wave in elongation.

In general we may say that the times of maxima of elongation are near the times of minima of cell division and *vice versa* in all plants studied. This reciprocal relation is not so clearly expressed as in the case where both processes may be observed at the same time and in the same individual cell as Ward (38) found in *Bacillus ramosus* Fraenkel and de Wildeman (39) found in Spirogyra; but is probably as near as might be expected from the fact that the two processes must be observed, not only in different cells, but also in different individual roots.

DISCUSSION

The question naturally arises: What are the causes of the rhythm found both in the elongation and the cell division of the plants studied? That it may be due to external influences of changes in illumination and temperature is out of the question, since this work was done in a dark room and the temperature was kept constant, except in a few cases, to within one degree. It seems quite clear, also, that it is not due to heredity, in the case of seedlings, as Semon (32) and Karsten (12) believed, since it has been shown by a number of earlier investigators that plants placed in continuous darkness and uniform temperatures gradually lose the periodicity which they had exhibited when exposed to the alternation of darkness and light. Now it would be expected that these rhythms would show some relation to the normal changes of night and day, even though the experimental plants were not so exposed, if the rhythms were due to the hereditary persistence of such effects upon the parent plants. It has been shown, however, in the case of *Pisum sativum* seedlings, that these rhythms have no relation to time of day, but rather that they depend, for the precise time of their appearance, upon the time of day when metabolic activity is initiated. It was at first thought that the rhythm might be due, in the case of germinating bulbs, to the persistence of a habit acquired by the bulb, while the bulb was itself growing and so exposed to the alternation of darkness and light, and the subsequent transfer of this habit to the growing parts. This is also disproved, since roots grown from seeds, in the case of *Allium Cepa*, exhibited the same rhythms as those grown from bulbs. That the rhythms of elongation and cell division may have a relation to the diurnal rhythms in atmospheric pressure and electrical potential is also out of the question, since it has been shown that the time of the waves in elongation and cell division depends upon the time of the initiation of metabolic activity, and that they vary according to the time when germination is begun, regardless of atmospheric conditions. Stoppel (34) found a relation existing between curves for sleep movements of plants and electrical potential of the atmosphere.

The two processes, growth and cell division, must necessarily go hand in hand as two of the vital activities of germinating seedlings. Just what the precise relation between them is, is not so definitely known, though it is quite evident that a certain size of the cell must be attained before cell division ensues, since cells from corresponding parts of different individuals of the same species vary but little in size. In a comparison of the curves for elongation and cell division it is seen that a general reciprocal relation exists between these two processes whereby there is a slowing-up in the rate of elongation at the time when there is the largest number of cells undergoing mitosis. The fact that the processes of elongation and cell division show such a reciprocal relation to each other within the individual cell is not so difficult to understand, since there is probably not enough energy available to permit both processes to go on at their maximum at the same time. It is to be recalled, however, that the zone of most extensive elongation in the root is not the same as the zone of mitotic activity (practically all mitoses occur within a zone bounded by the growing point and an imaginary line across the section back from the growing point a distance equal to twice the diameter of the root). This reciprocal relation between elongation and cell division in the root as a whole might be explained on the same basis as that in the individual cell, provided there is a coordination within the root tip sufficient so that when a large number of cells are undergoing mitosis the total energy available within the tip is directed more to mitosis than toward growth and elongation, and hence the one process will be near its maximum when the other is near its minimum. Whether it be a matter of available energy or not, the fact remains that the two processes, elongation and cell division, do alternate with each other, both in the individual cell and in the root as a whole. Since neither process can go on for any considerable length of time to the exclusion of the other, the curve representing the extent of either will show waves such as those found in the present work. Thus, activity once initiated by the beginning of germination of the seed or bulb, these two processes, of necessity having a definite relation to each other, bring about the rhythms here found.

The fact that these rhythms have a definite interval in the various series of the same variety of seedlings, and that corresponding waves in the different series bear the same relation to each other as the time interval between the times of initiation of metabolic activity, *i.e.*, that the maxima and minima in the different curves depend for the time of their appearance upon the time when germination was begun, indicates that the ultimate cause of this alternation between mitosis and elongagion is entirely an internal cause and not related to external conditions and is in perfect accord with the above suggested energy hypothesis. This harmony in the various series of plants of the same variety shows, further, that the rhythms here found are not mere chance variations in activity which, when plotted, show such curves, but rather that the two processes, elongation and cell division, follow each other in a regular manner, the root tip being occupied with one and then with the other, and hence showing a regular and definite oscillation from the one to the other.

Whether or not this reciprocal relation existing between elongation and cell division is sufficient entirely to account for these rhythms, and whether there might not also be other rhythms independent of, and more or less confused with, these first rhythms, is a question not satisfactorily answered by the data at hand. The fact that the times of maxima of elongation in a few cases did not coincide with the times of the minima of cell division might seem to indicate that there were other factors influencing the course of these activities in the plant besides the alternation of elongation and cell division. It is conceivable that a relation might exist between growth activity (including mitosis) and available food supply, whereby these metabolic processes might, once initiated, gradually increase and finally outweigh the capacity of the enzymes to render stored food available. Then, with a lessening proportion of available food, a slowing down of these processes must ensue until the food supply is again adequate, after which the same processes may be repeated. In other words, may there not be a certain inertia inherent in these vital processes, so that once they are in operation a certain force is required to check them, and, once slowed down, a certain force is again required to accelerate them? This might explain oscillations in either process independently of the other, or in the sum of the two processes, but it would not explain the reciprocal relation between the two processes. The possibility of growth rate exceeding that of enzymatic activity is apparent in the exhaustion effects found at higher temperatures in seedlings of *Zea Mays* and *Pisum sativum* by Lehenbauer (19) and Leitsch (20).

It is necessary, also, to distinguish between the terms "periodicity" and "rhythm." By "periodicity" the earlier workers meant a regular oscillation which was caused by the alternation of day and night or by other external changes, and which was lost when the environmental conditions were rendered constant; while the term "rhythm" in the present paper is restricted to mean any oscillation in activity which is definite and regular and not related to any external influences. Thus these roots in their development exhibit "rhythms" in the absence of changes in environment, but not a "periodicity" in the sense in which the older writers used the term.

SUMMARY

1. Under constant uniform conditions elongation in all plants studied proceeds in a rhythmic manner, two or more waves occurring during the 24-hour period.
2. Nuclear and cell division proceed in a similar rhythmic fashion.
3. The times of occurrence of maxima and minima are dependent upon the time of initiation of metabolic activity and not upon the time of day by the clock.
4. Elongation and cell division, as regards time of maxima and minima, are, in general, reciprocals of each other.
5. This reciprocal relation existing between elongation and cell division accounts for a large share, at least, of the rhythms found in these plants.

The writer desires to take this opportunity of expressing his appreciation to Professor F. C. Newcombe, under whose direction this work was done, for his constant encouragement and helpful criticism: also to Professor J. B. Pollock and Professor R. M. Holman for helpful criticism and suggestions.

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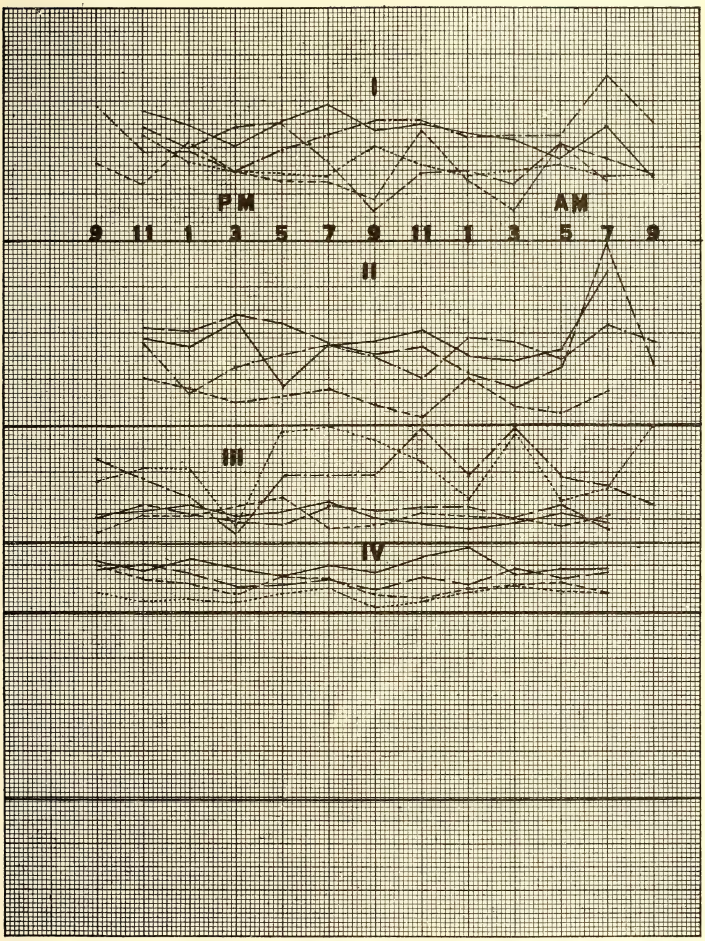

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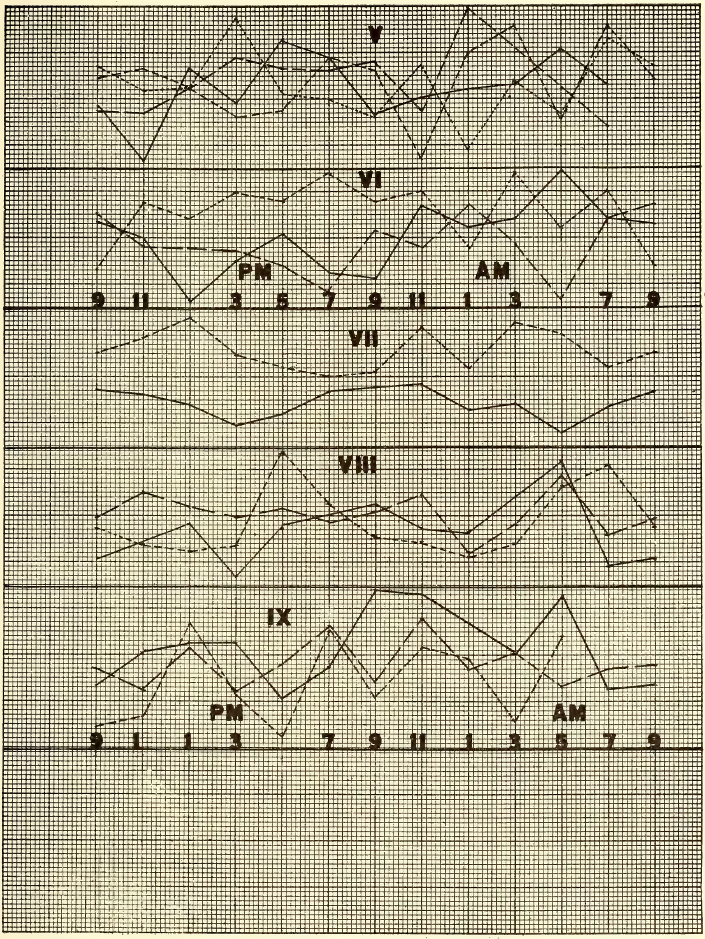

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EXPLANATION OF PLATES XXIV AND XXV

Ordinates in curves I–IV show rate of elongation in mm. per hour; in curves V–IX they show the number of cells per sq. mm. undergoing mitosis. The abscissae show time of day by the clock.

I

(Base line = 0. Scale, 1 square = .08 mm.)
193. (————) Elongation of *Pisum sativum*. Germination at 9 A.M.
194. (— — —) " " " " " " "
160. (– – – – –) " " " " " " 6 P.M.
174. (········) " " " " " " 8 P.M.
102. (— · —) " " *Zea everta*.

II

(Base line = 0. Scale, 1 square = .08 mm.)
70. (— — —) Elongation of *Lupinus albus*. Germination at 9 A.M.
73. (————) " " " " " " "
272. (– – – – –) " " *Allium Cepa* (bulb) " " "
296. (— · —) " " " " " " " "

III

(Base line = 0. Scale, 1 square = 0.1 mm.)
275. (————) Elongation of *Allium Cepa* (seed). Germination at 9 A.M.
276. (— — —) " " " " " " " "
288. (– – – – –) " " " " " " " "
111. (— · —) " " *Cucurbita Pepo* " " "
112. (········) " " " " " " "

IV

(Base line = 0. Scale, 1 square = .08 mm.)
273. (————) Elongation of *Allium Cepa* (seed). Germination at 9 A.M.
274. (— — —) " " " " " " " "
281. (– – – – –) " " " " " " " "
283. (········) " " " " " " " "

V

(Base line = 125. Scale, 1 square = 10 cells.)
2. (————) Mitosis in *Pisum sativum* (wrinkled) Germination 9 A.M.
27. (— — —) " " " " (smooth) " "
28. (– – – – –) " " " " " " 2 P.M.
31. (········) " " " " " " 8 "

VI

(Base line = 125. Scale, 1 square = 15 cells)
33. (————) Mitosis in Pisum (smooth). From refrigerator at 9 A.M.
35. (— — —) " " " " " " " 1 P.M.
7. (– – – – –) " " *Zea everta*. Germination at 9 A.M.

VII

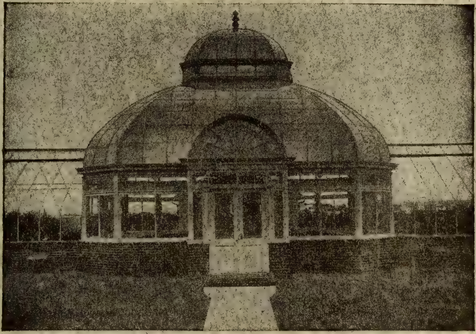
(Base line = 0. Scale, 1 square = 5 cells.)
1. (————) Mitosis in *Lupinus albus*. Germination at 9 A.M.
13. (– – – – –) " " " " " " "

VIII

(Base line = 50. Scale, 1 square = 10 cells.)
10. (————) Mitosis in *Allium Cepa* (bulb). Germination at 9 A.M.
24. (— — —) " " " " " " " "
5. (– – – – –) " " *Vicia faba*. Germination at 9 A.M.

IX

(Base line = 300. Scale, 1 square = 10.)
12. (————) Mitosis in *Allium Cepa* (seed). Germination 9 A.M.
22. (— — —) " " *Allium canadense* " "
23. (– – – – –) " " *Allium cernuum* " "



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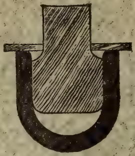

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THE MODIFICATION OF VEGETATIVE AND REPRODUCTIVE FUNCTIONS UNDER SOME VARYING CONDITIONS OF METABOLISM[1]

E. J. KRAUS

In virtually any text on the subject of plant physiology may be found paragraphs dealing more or less definitely or indefinitely with the functions of the so-called essential elements. Many of these treat of the relationships which specific elements or compounds have to the modification of reproductive or of vegetative functions, considering these either as separate entities or as mutually interdependent. Large numbers of contributions dealing with specific phases of the subject are constantly forthcoming from the fields both of research and of practice. Some of these are in the nature of deductions made largely on hypothetical grounds while others are based upon experiments of varied nature. The field which can be well covered by any investigator is limited, though the opportunity for constructive work is large. Much must be done in the way of assembling and interpreting the results of various investigations, especially in connection with the extended researches in chemistry, physics, and the related sciences on the one hand and with the practices of the applied sciences on the other.

Granting all this, it is self-evident that at this time we can scarcely do more than state the problem as it now seems to exist, and take a brief look at its possible future development. As time goes on it seems less and less possible to express any dogmatic opinions, or to draw any narrowly circumscribed conclusions from the data available.

Disregarding the notion that any circumstance which threatens the life of a plant causes such a plant to become markedly reproductive in order that the species may be perpetuated, one of the earliest attempts to explain, on a physiological basis, the apparently interrelated phenomena of vegetative extension and the differentiation of parts more intimately concerned in sexual reproduction, assumed that any of the higher green plants is in a state of adjustment between the materials which it derives from the soil and those substances which it manufactures from these ma-

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[1] Invitation address read before a joint session of the Botanical Society of America, the American Society for Horticultural Science, and the American Phytopathological Society at St. Louis, December 31, 1919.

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terials combined with elements from the air. These two classes of materials were sometimes spoken of as unelaborated and elaborated foods respectively. In general it was stated that if the unelaborated foods predominated, then the plant would tend toward the vegetative condition, whereas if the reverse were true, reproductive structures would be produced. Somewhat later this conception became more concise; nitrogen was considered as being the element among the unelaborated materials which was most effective in producing the vegetative condition, and the several types of carbohydrates were designated as the significant elaborated foods. Thus, in spite of the fact that certain experiments showed the fallacy of the notion, it was assumed that whenever leafy, succulent structures were desired, fertilizers containing nitrogen would produce the result, but that such fertilizers were to be withheld and others rich in potash and phosphorus supplied when fruit production was sought. The whole conception carried with it the idea that the two functions of vegetative extension and sexual reproduction were in some way antagonistic, and statements to that general effect may be found widely scattered throughout the literature of both botany and horticulture. Such an idea may have arisen from the general observation that when the expression of one or the other of these functions is reduced the other is apparently increased relative to an assumed average. Actually such a condition is generally unreal, for while it may be true that certain individual plants in a vigorously vegetative condition may produce fewer sexually-reproductive parts, it by no means follows that suppression of vegetation in itself will mean increased production of reproductive portions or fruitfulness. In fact it is quite possible to suppress reproduction and vegetation in direct relation one with another. In other words, it is not the mere decrease of vegetativeness that induces the production of parts concerned in sexual reproduction, nor the increase of sexual reproduction which decreases vegetative extension, but there is an underlying cause upon which each rests; both may be readily increased or decreased simultaneously, or one can be made to dominate the other through any one or more of several different means.

The recognition of this fact became general when clear-cut quantitative results of definite experiments showed that under certain circumstances the yields of fruit from the higher plants could be greatly increased when nitrogenous fertilizers were applied to them. More critical investigation and chemical analyses indicated that those plants which would respond in this manner were weakly vegetative and that their nitrogen content relative to their dry weight (or, as also shown, to their content of sugars and starch-like complexes) was very low. When the nitrogenous fertilizers were applied, however, the total relative nitrogen content of the plants increased, the plants becoming more strongly vegetative and more fruitful. These findings resulted in a third conception concerning the relationship of nitrogenous and carbohydrate compounds to vegetation and fruiting, namely, that if plants possess or are capable of synthesizing large carbohydrate reserves but available nitrogen is limited, then, when such nitrogen is supplied, vegetativeness and fruitfulness are both increased. It should be noted, however, that though the nitrogen content may be too low for fruiting, there is frequently an active and abundant differentiation of parts more particularly concerned in sexual reproduction such as buds, flowers, spores, and the like, in these carbohydrate-high plants, and that often such plants are not only more easily multiplied by vegetative means but actually tend more freely to produce specially modified, vegetatively reproductive structures.

On the basis of theory, it should be possible to conceive of at least one more relationship which might exist between the carbohydrate and nitrogenous materials, namely an abundant source of the latter but a meager supply or even a lack of the former. Such a condition actually does prevail at least for the non-saprophytic or non-parasitic higher plants when there is not sufficient light for synthesis of carbohydrates, or when these are limited or removed through insect attacks, pruning, or other agencies. The plants are weakly vegetative and non-reproductive. In the case last previously discussed, nitrogen and not carbohydrates constituted the limiting factor to growth and reproduction, and both were capable of being increased through the application of nitrogenous fertilizers; but in the instance now under consideration neither vegetativeness nor sexual reproductivity is increased by additions of nitrogen; it is only when conditions are provided such that carbohydrates may be formed in greater amount, or are directly supplied, or at least are not artificially removed, that the plants become first vigorously vegetative, and with opportunity for further increase and accumulation of the carbohydrates in relation to the nitrogenous nutrients they become sexually reproductive. Because of the failure clearly to differentiate between these two types of the weakly-vegetative non-reproductive condition, much of the conflict of ideas regarding the means by which vegetativeness or reproductiveness may be regulated seems to have arisen, but on the present conception it is an easy task to harmonize the results from many experiments which seemingly are at variance, or to explain why apparently the same practice may yield widely varying results

Categorically summarizing the foregoing considerations, on hypothetical grounds supported by a limited number of definite chemical analyses made on tomato and on some other species of plants, and on the basis of various suggestions by many workers, we have the following:

Class I. Though there be present an abundance of nitrogenous nutrients, with a low carbohydrate supply, vegetative extension occurs but slowly and sexual reproduction scarcely at all. There is complete or nearly complete absence of blossoms, vegetation is weak, the stems are very slender in comparison to their length and are soft and succulent with little woody tissue, the leaves are slender and light gray-green. Such plants are practically without the more complex reserve carbohydrates, and are high in moisture, in total nitrogen, and in nitrate nitrogen.

Class II. When there is present an abundance of nitrogenous nutrients and available carbohydrates, both are utilized in vigorous vegetative extension with little or no tendency toward sexual reproduction. The plants produce no sexual parts or a few frequently abnormal flowers or flower clusters which very often are partially transformed into leaves or stems, and very rarely set or mature fruit; they are exceedingly vigorous vegetatively, have stems of large diameter, soft and succulent with a small amount of woody tissue, and large, soft, and intensely dark green leaves. Such plants are relatively low in reserve carbohydrates but are higher than those of Class I, are high in moisture, in total nitrogen, and in nitrate nitrogen.

Class III. When there is a limitation of the nitrogenous nutrients in relation to the available carbohydrates, so that the latter can accumulate in excess of their utilization in vegetative extension, then the plants become sexually reproductive as well as vegetatively active. The plants produce many good-sized blossoms, a large proportion of which set and mature; are less vigorously vegetative than those of Class II; the stems are of large diameter but firm to the touch and with considerable woody tissue; the leaves are large, and dark to light green in color. Such plants contain greater quantities of reserve carbohydrates than those of Class II, but are lower in moisture, in total nitrogen, and in nitrate nitrogen.

Class IV. When there is a further relative reduction of the nitrogenous nutrients without inhibiting a possible increase of carbohydrates, there results a large accumulation of the latter, a decrease in vegetative activity and in sexual reproduction. Such plants produce few small-sized blossoms, a large proportion of which either fail to set and mature, or mature into small, tough fruits; they are feebly vegetative, the stems are of small diameter, very firm and hard to the touch with relatively much woody tissue; the leaves are small, stiff, and light green or yellowish in color. Such plants contain large quantities of the more complex carbohydrates, but are low in moisture and in total nitrogen and are almost completely lacking in nitrate nitrogen.

Naturally these classes, depending as they do upon a quantitative relationship of various substances within the plant, blend insensibly into one another according as these relationships are varied, but what might be called the mid-points within them are very distinct. What departures from these groups may occur when either light or temperature is made a limiting factor can not be stated, but they can be duplicated in soil, sand, or water culture. The term nitrogenous nutrients is used because at the present time it is not definitely known what forms the effective nitrogen may have within various plants. In some, great vegetative extension is associated with nitrogen in the nitrate form. Of course it is not assumed that carbohydrates and nitrogenous nutrients are the only compounds concerned in the varying expression of vegetative and reproductive functions. The results of recent experiments on the application of sulphur to certain leguminous crops, as well as those from the use of potash, phosphorus, and many other substances, would refute any such idea; they were considered in detail simply because there are available a large number of ponderable analyses concerning them and because they have long been favorite material for speculation.

To determine the rôle of water in respect to the varied plant functions is in itself a large problem. Not only must its direct effects in so far as it enters into chemical combination be deciphered, but a knowledge of its physical influences and of its direct consequences in the rendering available or non-available of other materials is also imperative. The effects of light and temperature, both on vegetation and on reproduction, will undoubtedly eventually find their clearest interpretation when studies have been made relative to the influence of these agencies on internal composition, and to how they are related to observed changes. How either light or temperature reacts upon the type and rate of water and salt absorption, upon the relative proportions of salts absorbed, and upon the processes of photosynthesis, metabolism, and storage, remains in large part still to be exactly determined. Then, too, what limits to the range of expression of any character, or function, or groups of characters or functions, are imposed by hereditary factors on the one hand, or by physiological factors on the other—if actually there is a possibility of separating them—remains for the geneticists, cytologists, and physiologists to determine.

From the physiological and morphological viewpoint, the several contributions by Klebs at once come to mind as being the most outstanding, both because of the range of forms investigated and of the number of environmental conditions considered. It is to be regretted that his work has not contributed a larger mass of data concerning the actual internal conditions and composition of the plants investigated. For the most part he has examined external conditions and external responses, and from the data thus obtained reasoned as to what might be the most probable internal situations and effective elements in producing the observed results.

Several other workers, however, have published analyses of plant tissues, such as the apple, the olive, and other species, on the basis of which it is possible definitely to correlate in a quantitative way the vegetative or reproductive tendencies of such forms with certain elements of their composition. There are also scores of recorded analyses of various plants which show the wide variations in composition of any particular species or variety at various stages of its development or maturity. It is unfortunate that many of these results are fragmentary and do not form a part of a series sufficiently long to admit of determining the range of effects of specific substances under a varied set of conditions. Nor are they sufficiently detailed, in many cases, to afford real clues as to the several forms in which an element may be present; a total nitrogen estimation alone, for example, is of very little significance in any attempt to determine the nitrogen metabolism of a plant. And yet, invaluable aid toward an interpretation of the problem under discussion can be gained by fitting together the records available, and many of the more recent contributions are very helpful. Specific quantitative measurements of substances frequently take on an entirely new significance when they are no longer considered by themselves alone, but rather in connection with other materials present, as ratios. When, for example, such suggestive results as have come from various experiments designed to determine the nutrient salt requirements of plants in various stages of development, under varied conditions of light and temperature and moisture, are finally coupled with analyses of the organic and inorganic materials in the plants themselves at these different stages of development, we shall have begun a genuine approach to the problem of metabolism.

At the outset of any experiment which concerns the functions of growth or of reproduction, it is quite as important to determine the condition, or better the composition, of the plants which are to serve as the basis for the investigation, as it is to know and control the external conditions imposed. Many of the apparently discordant results of various experiments are easily accounted for and harmonized when the composition of the material used as the basis for investigation is taken into account, or when the range of effects of any element is considered in connection with the limits imposed by other substances present. One has but to think of the effects of nitrogen as partially detailed previously, or of sulphur, or phosphorus, or other elements which enter into a vast number of organic compounds essential to growth, and of how they influence subsequent development when present in varying relative quantities.

At this time it is worth while to consider several points in connection with the analyses of tissues of plants and what these may show. The fact that plants require or absorb mineral salts in varying ratios, quantities, or proportions, means, in other words, that such absorption and utilization depend in considerable measure upon the composition of the plant itself, and will vary as such composition is varied. Changed or changing condition or expression is the external evidence of changed or changing composition. The living plant is constantly in a state of becoming adjusted to changing surroundings, it is the product of the interaction of all the elements of its environment. A change of any one of such elements requires a readjustment of the entire system unless such a change is at once offset by another which in its effects is antagonistic to it. If this is true, the necessity of possessing some facts or knowledge concerning the composition and the transformation of compounds in the plant in connection with those absorbed from the media surrounding it, is absolutely imperative. Anyone who has attempted such analyses can realize the difficulties to be encountered without being told about them. It is not sufficient to make determinations on whole plants *en masse*; the several parts must be considered separately in as minute detail as possible, and then all must be related to the whole. Particularly is this true in relation to leaves and stems, when investigating the carbohydrate situation in a series of tests. Brief reflection will render obvious the fallacies of judgment which are likely to arise, especially when samples are collected during the day following a period of sunshine. The speed of digestion of the more complex carbohydrates (if any have been synthesized) and of their translocation is widely variable depending upon the other nutrients present. The presence of equal quantities of polysaccharides, indicated by analyses of material taken at any particular moment, in itself could by no means be interpreted as indicating an equivalent rate of synthesis, utilization, or storage of such products. These latter points could be determined only by a long series of analyses under varying conditions, or through indirect methods quantitatively measuring respiration, carbon fixation, and the like. Caution must be observed, also, in attempting interpretations of the analyses of plants already in any particular state or condition, the cause of which it might be desired to determine; it is only through the knowledge of a *series* of effects that causes can be deduced. For example, an analysis of fruit buds on any kind of fruit tree made during the winter or early spring will not furnish sufficient evidence on which to formulate a theory as to the nutrient relations necessary for their differentiation or presence. Instead, a number of observations from spring to winter are essential, for it is more than probable that the conditions determining meristematic differentiation are quite different from those accompanying the further development of the parts in question after initial differentiation, and the conditions for flowering may not be those for fruit setting and development. In fact, if our proposals are of any value or are true, the conditions for the production of the various results can not be the same.

But it is unnecessary to dwell on the complications of the problem to the extent that it may seem too large even for the beginning of an attack. The question may be asked, how is any knowledge of the relationships of vegetation and reproduction significant to practice? In reply it may be stated that it is at the very foundation of the whole matter of plant production, for on it rests the real understanding of such problems as cultivation, fertilization, irrigation, propagation, pruning (regeneration), phases of disease control, and many others. It has been dangerously easy to suggest interpretations of many of the results from these various practices upon a hypothetical basis; but, lacking still an abundance of carefully worked out experimental evidence, little is to be gained from a mere theoretical consideration of the probable or possible points involved beyond establishing working bases. Certain points of attack become obvious at once to anyone really giving the problem thought. Even the working horticulturist, whether he be florist, vegetable gardener, or fruit grower, is constantly contributing excellent experimental evidence, and is more than eager for some rational means by which he can interpret it, at least to the point of knowing how to eliminate unprofitable practices on some fundamental basis. It is our duty and privilege to learn the effects of various practices, then to eliminate those which are mutually antagonistic to the gaining of any desired end and to utilize only those which are mutually supplementary. What could be more desirable from the economic standpoint, or, in other words, more practicable?

In conclusion, then, it seems that the most needed essential to further extension of knowledge on the effects of varying metabolic conditions on the modification of vegetative and reproductive functions is the coördination of our knowledge of external and internal conditions by those having the means, technique, and willingness to do this work. The working out and making available for study of the range effects of many more elements and compounds than the very few which are known at present is particularly desirable, so that it may be possible in the future to deal with tangible materials rather than with hypothetical proposals.

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THE CAMBIUM AND ITS DERIVATIVE TISSUES

III. A RECONNAISSANCE OF CYTOLOGICAL PHENOMENA IN THE CAMBIUM

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Introduction

In the second paper of this series, the writer (1920*b*) called attention to the fact that the dimensions and volume of the undifferentiated, actively dividing and growing cells of the lateral meristem or cambium vary greatly, not only in plants of different systematic affinities, but in different parts of a given individual and in stems grown under different environmental conditions. Therefore, the cambium provides an unusually favorable medium for the study of a number of fundamental cytological problems, particularly those of the "working sphere of the nucleus," the much discussed "nucleo-cytoplasmic relation," and phenomena of karyokinesis and cytokinesis in cells of unusual shapes and sizes.

Sachs (1893) held that, although plants vary enormously in their linear dimensions, their constituent cells are minute and of relatively uniform size. His student Amelung (1893) endeavored to prove, by means of an extensive series of measurements, that variations in the size of an organ or plant are due to differences in cell number rather than to fluctuations in cell size; a view which subsequently was championed on the zoological side by Conklin (1896, 1898), Rabl (1899), Driesch (1898, 1900), Schultz (1904), and others. Sachs (1892, 1893, 1895) and Strasburger (1893) concluded that the size of uninucleated cells, particularly of the undifferentiated cells of embryos and meristems, is determined by the "energizing" or "working sphere" of the nucleus, which they considered to be very restricted. Both investigators noted that unusually large or much elongated protoplasts tend to be multinucleate, and Sachs emphasized the fact that large, uninucleated cells either contain much passive (non-cytoplasmic) material or are relatively inactive until energized by the formation of numerous nuclei.

Are Cambial Initials Multinucleate?

The cambium is composed of elements of two distinct shapes and sizes. The ray initials commonly are roughly isodiametric and of the same general order of magnitude as the cells of embryos and terminal meristems. The initials which divide to form the elongated elements of the xylem and phloem, on the contrary, have one long and two short dimensions and are relatively large (figs. 47, 53). In gymnosperms and less highly differentiated dicotyledons, adjacent fusiform initials vary greatly in length and volume. The questions suggest themselves, accordingly: are the ray initials uninucleate and the elongated cells multinucleate, and are the striking variations in the length and volume of the fusiform initials closely correlated with fluctuations in the number of nuclei that are contained within them? Schacht (1856) and Russow (1882) were of the opinion that the elongated cells of the cambium contain more than one nucleus each, but Strasburger (1891) questioned the accuracy of their conclusions.

During the last few years, I have secured material of the cambium from a wide series of gymnosperms and angiosperms of both temperate and tropical regions. The specimens were removed from various parts of the stems, roots, and branches and from trees of different sizes and ages, and, in the case of certain species, were collected at frequent intervals throughout the growing and resting seasons. The tissues were transferred as rapidly as possible to various killing fluids, of which the chrom-acetic-urea solution proved to be the most effective. In none of this material have I found cambial initials which contained more than one nucleus each. As shown in figures 47 and 53, the elongated initials, in tangential, longitudinal sections of the cambium, frequently appear to be multinucleate, but this is due to the fact that several radially flattened cells (fig. 50) are exactly superimposed so that their nuclei lie close to the same focal plane. It is evident, accordingly, that the fusiform cells of the lateral meristem do not resemble other types of large or much elongated protoplasts, such as have been shown, by Schmitz (1879), Treub (1880), Johow (1880), Kallen (1882), Haberlandt (1887), Pirotta and Buscalioni (1898), Smolák (1904), Němec (1910), Lundegårdh (1914), and others, to be multinucleate. Each initial contains a single nucleus which is centrally located and retains this position during growth and karyokinesis. In other words, not only is there a much greater variability in the size of the meristematic cells than hypothesized by Sachs or Strasburger, but in the lateral meristem the nucleus may extend its "energizing" influence to a distance of several thousand microns. Strasburger found that the average diameters of the more or less isodiametric cells of terminal meristems varied between 5 and 24 microns. In certain gymnosperms, the cambial initials may attain a length of more than 9,000 microns and a volume of approximately 10,000,000 cubic microns.

Nucleo-cytoplasmic Relation

Strasburger's measurements led him to believe that there is a close correlation between cell size and nuclear size, a conclusion which was strongly supported by the experimental investigations of Gerassimow (1902), and which has led to considerable controversy among zoologists and botanists as to whether the so-called nucleo-cytoplasmic relation is a constant and self-regulating ratio. The painstaking investigations of a number of zoologists indicate that, although in general large cells tend to have larger nuclei than small ones, the nucleo-cytoplasmic ratio fluctuates within rather wide limits, not only in different organisms, but also during different stages in ontogeny and under different environmental conditions. Highly specialized tissue cells tend to have very different ratios from those characteristic of undifferentiated or embryonic elements.

TABLE 1. *Pinus Strobus* L.

	Nucleus				Cell				Ratio Between Volume of Nucleus and Volume of Cell
	Dimensions			Approximate Volume	Dimensions			Approximate Volume	
	VD	RD	TD		VD	RD	TD		
Cambium from 1-year-old stem									
	microns	*microns*	*microns*	*cu. mic.*	*microns*	*microns*	*microns*	*cu. mic.*	
Ray initials......	10.8	8.7	6.5	350	22.9	17.8	13.8	5,000	1:14
Large initials.....	63	3.2	5.8	1,000	870	4.3	16.	60,000	1:60
Cambium from 60-year-old stem									
Ray initials......	12.4	12.5	9.9	850	24.8	26.6	17.0	10,000	1:12
Large initials.....	82	5.9	8.9	3,500	4,000	6.2	42.4	1,000,000	1:286

Basis: dimensions of cells and nuclei are averages of 50 measurements.

The cambial initials of arborescent dicotyledons contain smaller nuclei (figs. 26, 40, 47) than do homologous cells of gymnosperms (figs. 1, 10, 33, 39, 42, 53). Whether this difference is due entirely to the fact that the cambial initials are smaller in the former than in the latter group of plants is a question which must be reserved for discussion in a subsequent paper. In Coniferae, as illustrated by *Pinus Strobus* L., the dimensions and volume of the nuclei vary considerably in cambial initials of different shapes and sizes. The large, fusiform initials have larger and more elongated nuclei than the small, roughly isodiametric, ray initials (figs. 1, 10, 14, and table 1). Furthermore, the large cambial initials of stout, mature stems tend to contain larger nuclei than do the small, meristematic cells of young shoots (table 1). However, as shown in table 2, the nuclei of fusiform initials are subject to variations in their longitudinal dimensions (VD) which are not closely correlated with fluctuations in the length of the cells. The most striking of these variations are seasonal. In the stem of the white pine, the "resting" nuclei of the fusiform initials tend to be much longer and narrower during the fall and winter than during the spring and summer (figs. 1, 10, and table 2). This change, if it occurs, is much less conspicuous in the root (fig. 33). It should be noted, in addition, that the variations in the shape of the nuclei are not paralleled necessarily by similar fluctuations in their volume. This is due to the fact that the changes in length may be more or less completely neutralized by concomitant changes in cross-sectional area.

TABLE 2. *Pinus Strobus* L.

Date	Source of Cambium			Nuclei		Fusiform Initials	
	Age of Tree, Years	Portion of Plant	Number of Rings Under Cambium	Dimensions (Microns)		Dimensions (Microns)	
				VD	TD	VD	TD
3/25/18	36	Stem	1	64	4.5	910	18.6
3/21/18	"	"	8	68	8.1	2090	23.7
3/9/18	"	"	27	67	8.9	2780	29.5
2/25/18	"	Branch	1	60	4.6	830	20.1
2/25/18	"	"	12	60	9.1	1950	27.7
4/22/18	67	Stem	1	56	4.3	800	16.3
3/24/18	"	"	48	82	8.9	3890	42.2
4/28/18	"	"	48	*41*	*17.3*	4000	38.5
3/24/18	"	"	61	84	7.9	3000	32.0
3/23/18	"	Large root	32	42	17.2	2760	40.5
3/2/18	25	Stem	1	56	6.1	890	16.3
3/2/18	"	"	15	57	7.9	2650	39.1
4/22/18*	"	"	19	71	6.3	1700	23.5
4/22/18*	"	"	19	*34*	*13.6*	1700	23.5
5/19/18	"	"	15	*36*	*15.3*	2400	35.3
6/17/18	"	"	17	*39*	*12.8*	3260	35.2
7/15/18	"	"	17	*32*	*16.3*	2970	36.8
8/19/18	"	"	17	*39*	*13.2*	2580	34.4
10/13/18	"	"	19	59	8.3	1710	29.8

Basis: Dimensions of cells and nuclei are averages of 50 measurements.

Italicized figures indicate that the nuclei are from cambial initials which have been dividing for some time previously.

* Both types of "resting" nuclei were present in the same sections, due to the fact that certain initials had divided, whereas others were still in the winter condition.

Although the values in table 1 indicate that the ratio between cell size and nuclear size may remain relatively constant in ray initials of different sizes, they show very clearly that this ratio varies greatly in fusiform initials of different dimensions and volumes. The nuclei do not elongate to any considerable extent as the fusiform initials increase in length. In other words, these meristematic cells do not contain very abnormally elongated nuclei, such as have been described and figured by Molisch (1899) for highly specialized tissue cells of certain monocotyledons. As previously stated, the relatively small, centrally located nucleus must, in certain cases, extend its energizing influence for several thousand microns in order to control processes of growth and longitudinal division in the cambial initials of various gymnosperms.

Boveri (1902, 1905) found that the size of the nuclei in echinoderm larvae is dependent upon the number of chromosomes which enter into the nuclei, and concluded that "die Grösse der Larvenzellen ist eine Funktion der in ihnen enthaltenen Chromatinmenge, und zwar ist das Zellvolumen der Chromosomenzahl direkt proportional." A few years later Gates (1909) showed that *Oenothera gigas*, a tetraploid mutation of *Oe. Lamarckiana*, is composed of larger cells than the species from which it originated. Similar phenomena have been observed by Gregory (1912), Winkler (1916), and others. The work of Gregory (1909) and Keeble (1912) indicates, however, that in certain giant forms of *Primula sinensis*, which have larger cells than the typical form, there is an increase in the size, but not in the number of chromosomes. Winkler's paper is particularly significant, in this connection, owing to his general discussion of the relation between cell size and chromosomal number in the higher plants. He reaches the conclusion that there is a very close correlation between cell size and chromosomal mass, both in meristematic and non-meristematic, somatic tissue. He states that in embryonic types of tissue (lateral and terminal meristems) the cells are roughly isodiametric, of nearly uniform size, and always contain the diploid number of chromosomes. In non-meristematic tissue, multinucleate protoplasts, nuclear fusions, and changes from the diploid to the tetraploid or the polyploid condition are of common occurrence, and many cells depart widely from the inherited, specific cell size of the plant. He infers that such cells tend to be hyperchromatic, much elongated elements containing more than one nucleus each and other types of large cells an abnormal number of chromosomes.

It is evident that the cambium provides a favorable medium for testing Winkler's generalizations. The fusiform initials, *which vary greatly in size*, obviously are not multinucleate. Are they hyperchromatic? Before attempting to answer this question it is essential to devote some attention to a discussion of karyokinesis in the lateral meristem.

KARYOKINESIS IN CAMBIAL INITIALS

As far as I have been able to determine, the nuclei of cambial initials always divide mitotically. Strasburger (1891) observed fragmentation in young sieve tubes of larch. My own preparations indicate that amitosis may occur in slightly differentiated cells of the cambial layer—which later are to develop into tracheary elements—just before the beginning of the growing season (fig. 38). Vacuolated nuclei also tend to be present at this time (fig. 38).[1] However, I have never seen any indication of either of these phenomena in undoubted *initials* of the lateral meristem of either gymnosperms or dicotyledons.

The cambium varies so greatly in its activities during different seasons, in different plants, and in different parts of given individuals, that the only rules which I have been able to formulate for obtaining division figures are, to collect specimens at frequent intervals, from as many individuals as possible, and from all parts of each plant. Initials may be dividing actively in one portion of a stem when those in adjacent portions are inactive. Similarly, numerous mitotic figures may be present in a given individual when a neighboring plant appears to be entirely devoid of them.

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[1] I am not entirely convinced that the phenomena observed by Strasburger and myself are not artifacts produced by poor fixation.

The karyokinetic figures in ray initials (figs. 15, 16, 30, 31, 32) generally resemble those that occur in parenchyma and terminal meristems. The figures vary considerably in fusiform initials, depending upon the shape and size of the nuclei and cells, and upon the plane in which the cells are dividing. Of course, the principal plane of division in elongated initials is periclinal or parallel to tangents to the circumference of the stem or root. In other words, the fusiform initials divide in a tangential, longitudinal plane which is a division plane of maximal area. Although the tangential diameter of the cambial initials increases to a certain extent during the earlier stages of enlargement of stems, roots, and branches (table 1), it falls far short of being sufficient to compensate for the rapid increase in the periphery of the cambium. Nägeli (1864) inferred from this fact that the fusiform initials must divide periodically in a radial, longitudinal plane. These hypothetical radial, longitudinal divisions are described and figured in many botanical textbooks, but I have been unable to find them in any of the gymnosperms and less highly specialized dicotyledons that I have studied. As shown by Robert Hartig (1895) and Klinken (1914)—for *Pinus sylvestris* L. and *Taxus baccata* L.—the fusiform initials elongate, sliding by one another, until they have attained a certain length. They then divide, by means of a more or less oblique, transverse partition into two short cells, which in turn elongate and divide. Thus, the increase in the periphery of the cambium is due primarily, not to radial, longitudinal divisions of the fusiform initials, accompanied by lateral enlargements of the products of such divisions, but to the sliding growth of periodically elongating and dividing cells. During the process of elongation, between successive pseudo-transverse divisions, the initials continue to divide in a tangential, longitudinal plane.

In dicotyledons (*e.g.*, Robinia) having short initials and small nuclei, the polar axis of the karyokinetic figure in a longitudinally dividing cell is placed at right angles to the main axis of the cell (fig. 25). In *Pinus Strobus* and other gymnosperms, on the contrary, it tends to assume a diagonal position during the late prophase, metaphase, anaphase, and early telophase (figs. 4, 5, 17, 18, 19). That this is not an artifact, due to the displacement of an ordinary spindle during fixation or sectioning, is indicated by the fact that the whole mitotic figure is asymmetrically developed in conformity with its oblique position (figs. 17, 18, 19). Furthermore, it should be noted that the radial diameter of the initials is so short (fig. 50) that there is not sufficient room to permit the elongated nucleus to shift into, or the karyokinetic figure to develop in, a transverse position. Of course, the position of the mitotic figure in pseudo-transversely dividing fusiform initials (figs. 7, 8, 9, 11, 12, 13, 28, 29) cannot be due to such factors as these, but is closely correlated with the orientation of the division membrane. When the partition is exactly transverse, the polar axis of the karyokinetic figure is parallel to the long axis of the cell (figs. 7, 9, 28, 29), but when it is oblique the division figure tends to assume a diagonal position (figs. 8, 11, 12, 13). In ray initials and in longitudinally dividing fusiform initials of *Pinus Strobus*, the chromosomes tend to be twisted and crowded together in the nuclear plate (figs. 4, 15), but in wide,[2] pseudo-transversely dividing fusiform initials, the nuclear plate may be more extensive and the chromosomes so arranged that they are nearly all visible in a single focal plane (figs. 8, 12). Furthermore, the chromosomes in adjoining cells may vary considerably in shape, in certain figures resembling hooks (fig. 12), and in others V's or U's of varying widths (figs. 7, 8, 15). The shape of the karyokinetic figures, particularly during the prophase, is profoundly affected by the shape of the "resting" nuclei. Thus, the spirems formed in the stem at the beginning of the growing season, when the nuclei are much elongated (figs. 10, 11, 41), may be entirely unlike those which are formed subsequently from shorter and wider nuclei (figs. 1, 6).

NUCLEO-CYTOPLASMIC RELATION (*continued*)

In order to determine whether large meristematic cells are hyperchromatic, I have devoted considerable attention to a study of the number and size of the chromosomes in the cambium of *Pinus Strobus* L. Sections were secured showing adjacent meristematic cells of various dimensions and volumes in equivalent stages of karyokinesis. The large, fusiform initials do not contain the tetraploid or polyploid number of chromosomes. All cells regardless of their size have approximately the diploid number (24).[3] It is evident, accordingly, that the variations in the size of the nuclei and cambial initials of *P. Strobus* are not dependent upon variations in the number of chromosomes. Are they correlated with fluctuations in the size of chromosomes?

Erdmann (1908) concluded from her investigations upon sea urchins that chromosomal mass, rather than chromosonal number, is the size-determining factor of cells. Conklin (1912) considered that in comparable cells of Crepidula large protoplasts have larger nuclei and chromosomes than small protoplasts. Hegner (1920) reaches similar conclusions in a recent paper upon Arcella. If there is a close correlation between chromosomal mass and volume of cytoplasm, the adjacent cells of the cambium vary so greatly in volume that there should be a striking contrast in the size of their chromosomes. This does not appear to be the case, however, in any of the material that I have studied. The chromosomes in a small ray initial having a capacity of 3,000–10,000 cubic microns may be fully as long and thick as those which occur in an adjoining fusiform initial with a volume of 1,000,000–5,000,000 cubic microns. Figures 4, 5, 17, 18, 12, 13, 28, and 29 are from fusiform initials having capacities of 800,000–1,200,000 cubic microns, and figures 15, 16, 30, and 31 from ray initials with volumes of 5,000–10,000 cubic microns. The chromosomes in figure 12 appear to be longer than those in figures 4 and 15 because they are flat, hook-shaped, and visible in one focal plane, whereas those in the other figures are U-shaped, twisted, and in different focal planes. Of course, as admitted by Conklin, it is difficult to determine the volume of individual chromosomes with any considerable degree of accuracy, but comparisons between figures 4, 7, and 15; 5, 13, and 16; 17, 28, and 30; and 18, 29, and 31 indicate very clearly that the large, fusiform initials of the lateral meristem are not hyperchromatic. The striking variations in the size of the nuclei (figs. 10 and 14) are due to differences in the volume of their achromatic portions. Thus, the volume of nucleolar matter is much greater in the large nuclei. Furthermore, the staining reactions of the "resting" nuclei suggest that the chromatin is more concentrated in the smaller than in the larger nuclei. In chromatin stains, the small nuclei of ray initials are very heavily overstained long before the large nuclei of adjoining fusiform initials become clearly differentiated.

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[2] Tangential dimension.

[3] Miss Ferguson (1904) has shown that the haploid number is 12.

It is evident, accordingly, that, although in certain cases variations in the volumes of cells are closely associated with fluctuations in the number of chromosomes (Boveri, Gates, Winkler) and in others with fluctuations in the size of chromosomes (Erdmann, Gregory, Keeble, Conklin, Hegner), the undifferentiated, actively dividing cells of the lateral meristem may vary greatly in size without corresponding variations in chromosomal size or number.

CYTOKINESIS

Certain of the fusiform initials in Coniferae are several hundred times as long as they are wide (radially), yet they divide longitudinally. What then is the nature of cytokinesis in cells of such extraordinary dimensions? During the telophase (fig. 17) the central spindle expands laterally by the addition of peripheral fibers and gradually assumes the form of a more or less warped disk (fig. 18). The connecting fibers, and later the accessory fibers, thicken to produce a cell plate in the usual manner, and then the fibers disappear except for a circular rim of kinoplasm. In tangential, longitudinal sections of the cambium, this ring-shaped aggregation of kinoplasmic fibrillae forms a halo about the daughter nuclei (fig. 56).[4] The ring increases in circumference by the addition of new peripheral fibers and extends the cell plate as it does so. When it intersects the radial walls of the cell, it becomes more or less four-sided (fig. 57). As soon as the cell plate comes in contact with the radial facets of the protoplast, the kinoplasm disappears from two sides of the frame-like figure, leaving two entirely separate aggregations of kinoplasmic fibrillae, which are parallel and which cross the cell from one radial wall to the other (fig. 58). These rod-like masses of kinoplasm (kinoplasmasomes) move in opposite directions, thereby extending the cell plate towards the ends of the cell. In radial, longitudinal sections of the cambium, the kinoplasmasomes are seen in section (figs. 20, 21). They are located midway between the tangential facets of the cell and usually are equidistant from the daughter nuclei or approximate center of the protoplast. This indicates, of course, that they move forward at equal rates. As shown in figure 22, they have a wedge-shaped outline, bluntly convex in front and tapering to a point at the rear along the cell plate. They are composed of fibrillae which resemble those that occur in the spindle. The threads or "lines of flow" do not extend across the cell from one tangential membrane to the other, but lie free in the cytoplasm. Nor are they connected with the daughter nuclei, which remain in their original position near the center of the protoplast. As new peripheral fibers are successively added in front, those at the rear disappear from about the recently formed portion of the cell plate (fig. 22). The latter is gradually extended in this singular fashion, often for a distance of several thousand microns, until it eventually reaches the ends of the protoplast; thus dividing the latter into halves, each of which contains one of the daughter nuclei. The nuclei become enclosed in a nuclear membrane and reform their nucleoli long before the kinoplasmasomes reach the ends of the cell.

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[4] Beer and Arber (1915) reached the conclusion that binucleate cells are of common occurrence in the growing tissues of the higher plants. They state: "The nuclei of the multinucleate cells generally arise by mitosis, but there are certain exceptional features connected with this mitosis and with the behavior of the associated protoplasm. The most striking of these is that two daughter-nuclei in the telophase, between which no wall-formation is in progress, are often found enclosed in a hollow sphere of dense and deeply staining protoplasm, the appearance at first glance suggesting a cell within a cell." I strongly suspect that the phenomenon referred to by them is a phase of cytokinesis not unlike that illustrated in figure 56.

Similar phenomena occur in dicotyledons during longitudinal division of the fusiform initials (figs. 23, 24, 25), but, owing to the smaller size of the cells, the kinoplasmasomes and cell plates are smaller than in the Coniferae. The oblique partitions of fusiform initials, and the longitudinal and oblique divisions of their derivative cells, are also formed by the intervention of these extraordinary cell plates (figs. 27, 51, 54, 55). Furthermore, the writer has accumulated considerable evidence which indicates that the phenomena in question are not confined to the cambial layer, but that they occur in other somatic tissues of the higher plants, in elongated or much flattened cells whose planes of division have one long and one short dimension.

Types of Cell Plate Formation in the Higher Plants

It is of interest to inquire what relation this type of cell plate formation —which is so greatly extended, both as regards space and time, and so clearly dissociated, except in its initial stages, from the usual phenomena of karyokinesis—bears to those types which previously have been described by Treub (1878), Strasburger (1880), and Schürhoff (1906). In Treub's first type, the nucleus is centrally located and the central spindle merely expands symmetrically until it touches the four walls of the isodiametric cell. In his second type, the nucleus lies near one wall and the daughter nuclei and "fibrillar-complex" migrate to the opposite side during cytokinesis. Strasburger described a third type, in which the daughter nuclei remain on one side of the cell and the "fibrillar-complex" crosses it. Schürhoff found a fourth type, in large cells having centrally located nuclei and small spindles. It differs from the preceding types in that "mit der Neubildung der peripheren Cytoplasmafäden geht die Auflösung der zentralen Strahlungen Hand in Hand." [5] In other words, a ring or halo of kinoplasm is formed such as occurs in cambial initials during incipient stages of cytokinesis.

A comparative study of cell plate formation in different somatic tissues, and in cells of different shapes and sizes, suggests that the various types of phenomena, described by Treub, Strasburger, Schürhoff, and the writer, are but different phases or stages of a single general or fundamental type of cytokinesis. The particular expressions of the phenomenon which may occur in any given cell are dependent upon its dimensions, its plane of division, and the size and location of the nucleus. Thus, in very small, isodiametric cells having a large, centrally located nucleus, the cell plate quickly intersects the walls of the cell without any extensive lateral enlargement of the central spindle. In larger cells with small nuclei, there is sufficient room for the process of cytokinesis to reach the halo or frame stage before the cell plate intersects the sides of the cell. However, only in elongated or much flattened elements is it possible for the phenomenon of cell plate formation to pass through the spindle, disk, halo, and frame stages, and finally to form two entirely separate aggregations of kinoplasmic fibrillae, kinoplasmasomes.

All five types of cytokinesis may be found in the cambium. Treub's first type occurs in ray initials (fig. 31) and in transversely dividing fusiform initials (figs. 29, 49). Schürhoff's type also is present in certain ray initials (fig. 32) and transversely dividing fusiform initials (fig. 48). Although the nuclei are centrally located under normal conditions, I have found them in a lateral position in Sequoia, in fusiform initials which were dividing to form callus. During cytokinesis the daughter nuclei remained on one side of the cell, as in Strasburger's preparations (figs. 45, 46), or one or both of them migrated across the cell, as in Treub's second type of cytokinesis (fig. 52).

Significance of Cytokinesis in the Cambium

The formation of the cell plate in cambial initials promises to be significant in the discussion of a number of fundamental cytological and physiological problems.

Strasburger's (1875, 1880, 1882) suggestion that spindle fibers are of cytoplasmic origin and his conclusion that the cell plate originates in swellings of the connecting spindle fibers have been questioned by various investigators. Treub (1878) held that the cell plate arises from free cytoplasmic granules which migrate into the equatorial region of the spindle, and Zacharias (1888) contended that the spindle is of nuclear, and the cell plate of cytoplasmic, rather than of kinoplasmic, origin. Mottier (1900) found that in certain of the higher plants the connecting fibers do not thicken appreciably in the equatorial region, and do not lie sufficiently close together to enable the slightly thickened middle parts to meet and fuse. He concluded from this "that the cell plate is formed by a homogeneous plasma which is conveyed to the cell plate region and deposited there by the connecting fibers." Strasbürger's generalization concerning the kinoplasmic origin of the cell plate was ably defended by Timberlake (1900), who concluded, however, that the nucleus "is the center of metabolic processes concerned in the production of the kinoplasm."

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[5] A phenomenon previously noted by Went (1887).

Strasburger (1880) and Went (1887) noted that the connecting spindle fibers appear to increase in number prior to the formation of the cell plate, and the former investigator subsequently suggested that this increase might be due to a splitting of the original fibrillae. Timberlake endeavored to prove that the lateral expansion of the spindle is not due to the formation of new fibers, but to the separation and enlargement of the original connecting fibers, coupled in certain cases with the addition of peripheral threads, formed from fibers which radiate from the daughter nuclei.

Hof (1898) and Němec (1899) showed that after the cell plate is complete the connecting fibers appear to be "drawn in" toward the cell plate and their place taken by granular cytoplasm. Timberlake stated that "concurrent with the growth of the cell plate in extent and the disappearance of the fibers from a central portion of the spindle the nuclei come to lie nearer the cell plate." He concluded that "all of the fibers which form cell plate elements are completely used up in the growth of the cell plate," and that "during the period of growth the cell plate may so shift its position as to lie in a plane different from that in which it was first formed."

The formation of the cell plate in cambial initials is significant in these connections. There is an enormous increase in the number of "peripheral" fibers during cytokinesis. As in the case of the radial fibrillar systems, which Harper and Dodge (1914) have shown to function in the formation of the capillitium of certain Myxomycetes, the kinoplasmic fibers have no visible connection with the nuclei. They are so far removed from the nuclei (figs. 20, 21) during the later stages of cytokinesis that one is inclined to question Timberlake's generalization concerning the nuclear origin of kinoplasm. The phenomena appear rather to justify Strasburger's contention that the accessory fibers are of cytoplasmic origin. As the original connecting fibers "draw in" or disappear, the nuclei tend to move towards the cell plate (figs. 19, 32, 45, 55), but subsequently may move away from it (figs. 21, 27). That the connecting fibers may thicken considerably in the equatorial region of the spindle is shown in figure 30; but a discussion of the finer details of cell plate formation is reserved for a subsequent paper. The width of the kinoplasmasomes closely approximates that of the central spindle. In other words, broad kinoplasmasomes tend to occur in cells having large karyokinetic figures (figs. 27, 29) and narrower ones in elements having smaller spindles (figs. 17, 18, 19, and 20; figs. 25, 24, and 23, and figs. 31 and 32).

The phragmoplasts develop along straight lines or curves depending upon the orientation of the karyokinetic figure and the plane in which the initial is dividing. In longitudinally dividing fusiform initials, in which the polar axis of the karyokinetic figure is transverse (fig. 25), the kinoplasmasomes move toward the ends of the protoplast along its longitudinal axis (figs. 23 and 24). When the mitotic figure occupies a diagonal position (fig. 17), the phragmoplast, during its earlier stages, curves toward the center of the protoplast avoiding the tangential facets of the cell (figs. 18, 19), and subsequently straightens out as shown in figures 20 and 21. In obliquely dividing cells having diagonally oriented division figures, either the phragmoplast develops in a single plane (figs. 27, 51, and 52), or the kinoplasmasomes meander more or less, forming undulating or curved partitions (fig. 54). In the formation of new ray initials, which are carved out of fusiform initials, both kinoplasmasomes may, in certain cases, curve toward and intersect the same radial surface of the cell.

Thompson (1917) is of the opinion that Errera's (Plateau's) law of minimal area is not invalidated by the occurrence of oblique or curved partitions provided these membranes are sigmoid and intersect the older walls at right angles. De Wildeman (1893) found that in certain cells the polar axis of the division figure shifts to a diagonal position previous to the formation of the cell plate, which curves during its development so as to intersect the sides of the cell at right angles. That all diagonal partitions do not develop in this manner is indicated very clearly by the phenomena in the cambium. The writer (1920*a*) has shown that the kinoplasmasomes, cell plates, and young membranes intersect the sides of the cell at varying degrees of acuteness, as well as at right angles. This suggests, of course, that in dealing with cytokinesis we are not concerned with protoplasm in liquid or semi-liquid phases. In other words, cellular membranes, at the moment of their formation, frequently do not assume the forms which would be assumed, under similar conditions, by liquid films destitute of weight (Plateau).

NUCLEOLI

The cambium of Coniferae appears to be an unusually favorable medium for the study of the structure and function of nucleoli, which are unusually large and conspicuous in most representatives of the family (Pls. XXVI, XXVII). In *Pinus Strobus* they vary greatly in size, shape, and number in different initials (figs. 1, 10, 14, 33). They are clearly visible during the early prophases (figs. 6, 41) and late telophases. Many of my preparations suggest that the large nucleoli are composed of aggregations of smaller nucleolar masses (fig. 43). During the process of aggregation hollow spheres (fig. 44) may be formed which resemble "vacuolated" nucleoli. The larger nucleoli also appear to fuse in many cases (figs. 1, 33, 41), particularly in cells which are differentiating into tracheary elements or sieve tubes (fig. 35*a*).

If nucleoli actually are concerned in the growth or enlargement of chromosomes, as is maintained by various botanists, one might expect the nuclei of fusiform initials (fig. 10), which contain a relatively large volume of nucleolar matter, to form much larger chromosomes than the small nuclei of ray initials (fig. 14). This is not the case, however, in *Pinus Strobus*, as I have shown on preceding pages.

There is a striking contrast between the cambial nucleoli of gymnosperms and those of dicotyledons. The latter tend to be surrounded by a conspicuous halo (fig. 40), which is absent in the Coniferae (Pls. XXVI, XXVII). Of course, this halo is supposed to be an artifact (Nägeli), yet the fact that it occurs so constantly in one group and not in the other suggests that it may be significant from the morphological or the physicochemical point of view.

Schwarz (1884) and Zacharias (1895) noted that during the differentiation of tissue elements in the growing points of plants, the volume of the nucleoli, as of the nuclei, first increases and then decreases. Similar phenomena occur in the cambial zone of *Pinus Strobus*. During the early stages of the differentiation of tracheids and sieve tubes, the nuclei become coarsely granular. The nucleolar mass subsequently decreases (figs. 35, 36, 37), and the nucleus becomes profoundly modified in shape and loses its staining capacity (figs. 36, 37). As suggested by Strumpf (1898), the crumpling of the nucleus (fig. 37) in young sieve tubes may be mistaken for fragmentation.

In conclusion, it is to be emphasized that this paper merely presents the results of a reconnaissance of cytological phenomena in the cambium. Its primary object is to outline salient features of the more striking phenomena encountered, and to pave the way for subsequent and more detailed investigations, on the part both of the writer and of others who may become interested in the cambium as an unusually favorable medium for the study of certain cytological and physiological problems.

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Summary and Conclusions

1. The initials of the lateral meristem or cambium, which may attain a length of more than 9,000 microns and a capacity exceeding 10,000,000 cubic microns, are uninucleate. The working sphere of their nuclei must extend in certain cases for a distance of several thousand microns.

2. The nucleo-cytoplasmic ratio may be relatively constant in ray initials, but varies enormously in fusiform initials.

3. All of the cambial initials of *Pinus Strobus*, regardless of variations in size, contain the diploid number of chromosomes.

4. Small ray initials having a capacity of 5,000–10,000 cubic microns may contain as large chromosomes as adjacent fusiform initials with a volume of 1,000,000–5,000,000 cubic microns.

5. Fusiform initials, which frequently are several hundred times as long as they are wide, divide longitudinally by an extraordinary extension of the cell plate.

6. The various types of cell plate formation that have been described by Treub, Strasburger, Schürhoff, and the writer appear to be merely different phases or stages of a single general type of cytokinesis.

7. The significance of the cambium in the investigation of various cytological problems, particularly of those relating to the cell plate and the dynamics of cytokinesis, is briefly discussed.

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DESCRIPTION OF PLATES

Plate XXVI

Fig. 1. *Pinus Strobus* L. "Resting" nucleus from fusiform initial, showing characteristic shape during spring–summer. Tangential, longitudinal extension. × 850.

Fig. 2. Same. Nucleus from longitudinally dividing, fusiform initial, seen in radial, longitudinal extension. Early prophase. × 850.

Fig. 3. Same. Later prophase. × 850.

Fig. 4. Same. Oblique position of karyokinetic figure, metaphase. × 850.

Fig. 5. Same. Early anaphase. × 850.

Fig. 6. Same. Karyokinetic figure from transversely dividing, fusiform initial, seen in tangential, longitudinal extension. Spirem formed by short, wide type of resting nucleus. × 850.

Fig. 7. Same. Metaphase from transversely dividing, fusiform initial, seen in tangential, longitudinal extension. × 850.

Fig. 8. Same. Metaphase from wide, obliquely dividing, fusiform initial, seen in tangential, longitudinal extension. × 850.

Fig. 9. Same. Late anaphase from narrow, transversely dividing, fusiform initial, seen in tangential, longitudinal extension. × 850.

Fig. 10. Same. "Resting" nucleus from fusiform initial, showing characteristic elongated, fall–winter shape. Tangential, longitudinal extension. Compare figure 1. × 850.

Fig. 11. Same. Elongated type of spirem formed by elongated nucleus. Compare figure 6. × 850.

Fig. 12. Same. Metaphase from obliquely dividing fusiform initial, seen in tangential, longitudinal extension. × 850.

FIG. 13. Same. Anaphase from obliquely dividing fusiform initial, seen in tangential, longitudinal extension. × 850.

FIG. 14. Same. "Resting" nucleus in ray initial. × 850.

FIG. 15. Same. Metaphase in ray initial. × 850.

FIG. 16. Same. Anaphase in ray initial. × 850.

PLATE XXVII

FIG. 17. *Pinus Strobus.* Early telophase from longitudinally dividing fusiform initial, seen in radial, longitudinal extension. × 850.

FIG. 18. Same. Widening of central spindle and beginning of cell plate formation. Longitudinally dividing fusiform initial, seen in radial, longitudinal extension. × 850.

FIG. 19. Same. Later stage than that shown in figure 18, showing disappearance of connecting fibers and movement of nuclei toward the cell plate. × 850.

FIG. 20. Same. Later stage than that shown in figure 19, showing daughter nuclei, cell plate, and kinoplasmasomes. × 850.

FIG. 21. Same. Later stage than that shown in figure 20, showing movement of kinoplasmasomes in opposite directions toward ends of protoplast and daughter nuclei in original positions at center of cell. × 400.

FIG. 22. Same. Section of kinoplasmasome showing fibrillæ and cell plate. × 2000.

FIG. 23. *Robinia Pseudo-Acacia* L. Fusiform initial in radial, longitudinal extension, showing cell plate, kinoplasmasomes, and daughter nuclei. × 850.

FIG. 24. Same. Less advanced stage of cytokinesis. × 850.

FIG. 25. Same. Beginning of cell plate formation. Note transverse position of polar axis of division figure. × 850.

FIG. 26. Same. "Resting" nucleus of fusiform initial, seen in radial, longitudinal extension. × 850.

FIG. 27. *Pinus Strobus.* Cytokinesis in obliquely dividing fusiform initial, seen in tangential, longitudinal extension. One daughter nucleus out of plane of section. × 850.

FIG. 28. Same. Early telophase in transversely dividing fusiform initial, seen in tangential, longitudinal extension. × 850.

FIG. 29. Same. Later phase than that of figure 28, showing extension of spindle and beginning of cell plate formation. × 850.

FIG. 30. Same. Thickening of connecting fibers in equatorial region of spindle. Ray initial. × 850.

FIG. 31. Same. Later stage than that shown in figure 30, showing cell plate. × 850.

FIG. 32. Same. Later stage than that of figure 31, showing extension of cell plate and "drawing in" of fibers and nuclei. × 850.

Figures 2, 3, 4, 5, 17, 18, 19, 20, and 21 of Plates XXVI and XXVII illustrate successive stages of karyokinesis and cytokinesis in a longitudinally dividing fusiform initial of a conifer (radial, longitudinal extension). Figures 15, 16, 30, 31, and 32 illustrate a similar series for a ray initial, and figures 26, 25, 24, and 23, a series for a short fusiform initial of a highly specialized type of dicotyledon.

PLATE XXVIII

FIG. 33. *Pinus Strobus.* "Resting" nucleus from fusiform initial of root, showing shape during winter. Contrast figure 10. × 850.

FIG. 34. Same. Nucleus from fusiform derivative cell which is in the earliest stages of differentiation into tracheary element. × 850.

FIG. 35. Same. Later stage than that of figure 34. × 850.

FIG. 35*a*. Same. Fusion of large nucleoli. × 850.

FIG. 36. Same. Later stage than that shown in figure 35. × 850.

FIG. 37. Same. Nucleus from young, differentiating sieve tube. × 850.

FIG. 38. Same. Fragmentation of nucleus and nuclear vacuoles in derivative cells of cambium. × 450.

FIG. 39. *Juniperus virginiana* L. Nucleus from fusiform initial seen in tangential, longitudinal extension. × 850.

FIG. 40. *Sassafras officinale* Nees et Eberm. Nucleus from fusiform initial, showing halos about nucleoli. Tangential, longitudinal extension. × 850.

FIG. 41. *Pinus Strobus.* Nucleus from fusiform initial, showing early prophase and nucleoli. Tangential, longitudinal extension. × 850.

FIG. 42. *Cedrus libani* Barrel. Nucleus from fusiform initial, showing nucleoli. Tangential, longitudinal extension. × 850.

FIG. 43. *Pinus Strobus.* Aggregating nucleolar masses. × 2000.

FIG. 44. Same. Vacuolated nucleoli. × 2000.

PLATE XXIX

FIG. 45. *Sequoia sempervirens* Endl. Cytokinesis in fusiform initial which is dividing to form callus. × 850.

FIG. 46. Same. Later stage than that of figure 45. × 850.

FIG. 47. *Robinia Pseudo-Acacia.* Tangential, longitudinal section of cambium, showing ray and fusiform initials. × 110.

FIG. 48. *Pinus Strobus.* Cytokinesis in transversely dividing, fusiform initial. Tangential, longitudinal extension. × 850.

FIG. 49. *Sequoia sempervirens.* Cytokinesis in transversely dividing fusiform initial. Tangential, longitudinal extension. × 850.

FIG. 50. *Pinus Strobus.* Transverse section of cambium and adjoining xylem and phloem. × 110.

FIG. 51. *Sequoia sempervirens.* Cytokinesis in obliquely dividing fusiform initial. Tangential, longitudinal extension. × 500.

FIG. 52. Same. Cytokinesis; daughter nuclei have migrated to opposite sides of cell. × 500.

FIG. 53. *Pinus Strobus.* Tangential, longitudinal section of cambium, showing two types of initials. × 110.

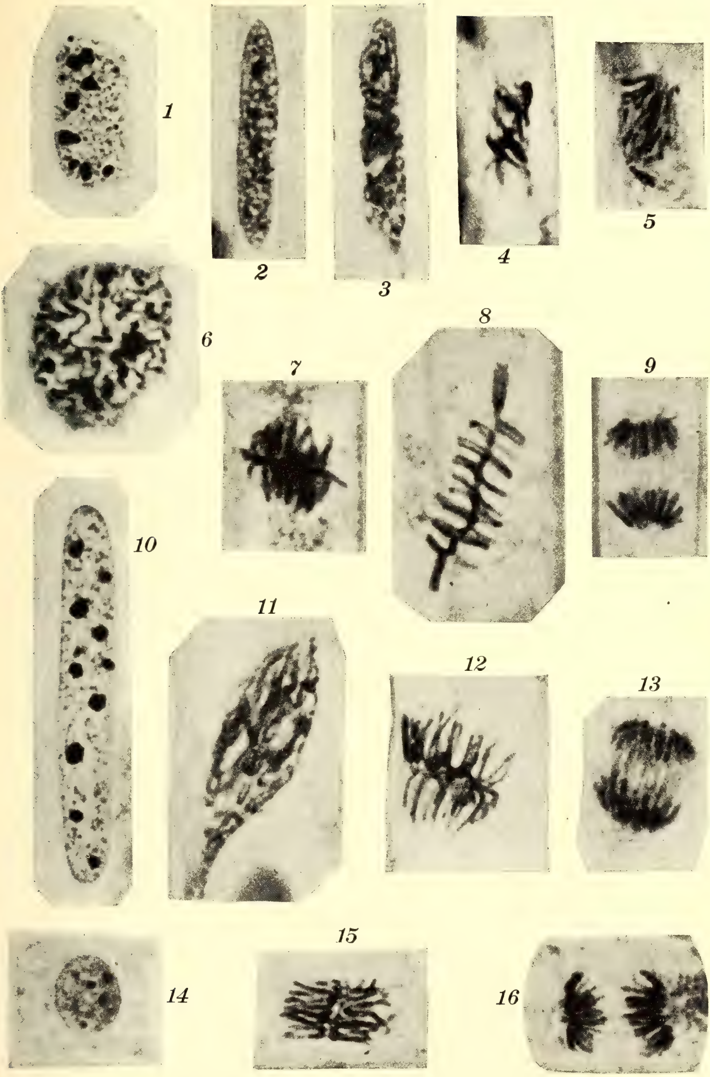
FIG. 54. Same. Cytokinesis in derivative cell of cambium, showing curvature of cell plate. × 500.

FIG. 55. Same. Cytokinesis in obliquely dividing fusiform initial, seen in tangential, longitudinal extension. × 500.

FIG. 56. Same. Halo or "cell within cell" stage of cytokinesis in longitudinally dividing fusiform initial, seen in tangential, longitudinal extension. × 500.

FIG. 57. Same. Later stage than that shown in figure 56. × 500.

FIG. 58. Same. Later stage than that of figure 57, showing kinoplasmasomes. × 500.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BAILEY: CYTOLOGICAL PHENOMENA IN CAMBIUM.

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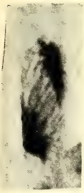

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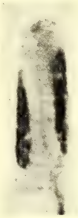

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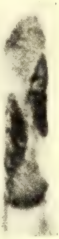

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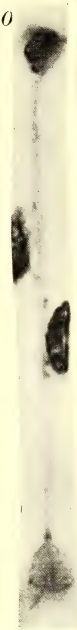

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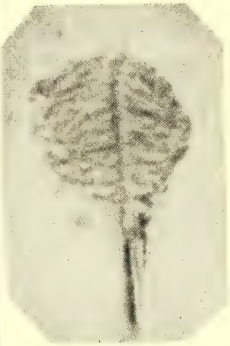
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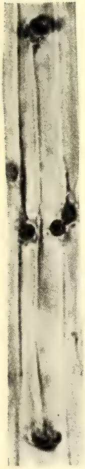

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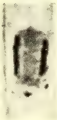

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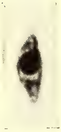

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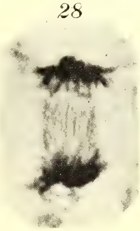

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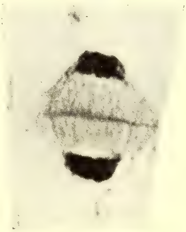

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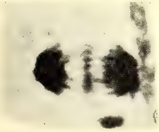

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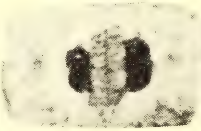

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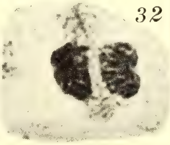

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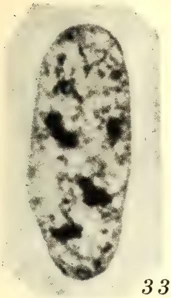
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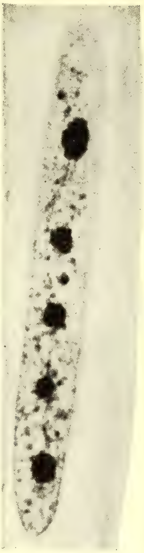
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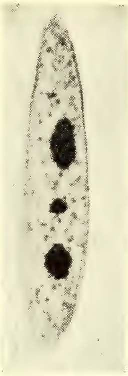
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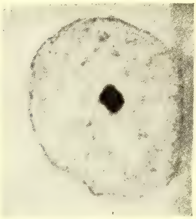
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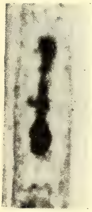
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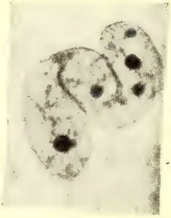
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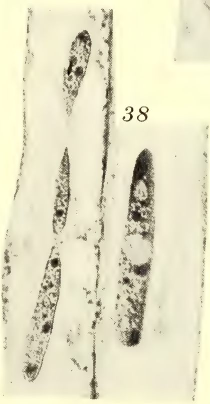
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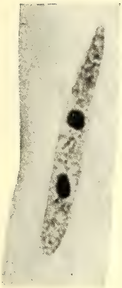
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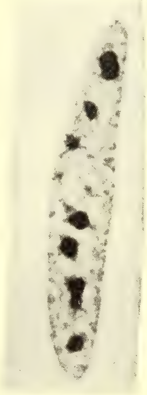
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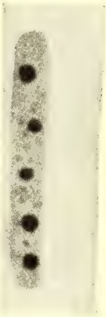
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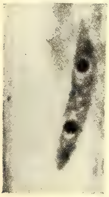
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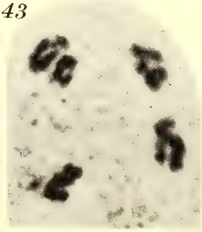
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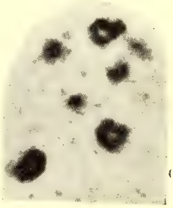
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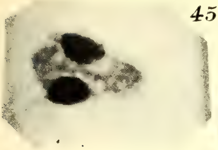
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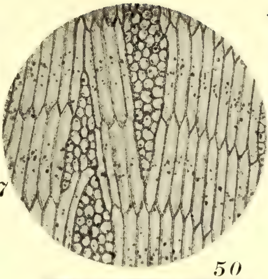
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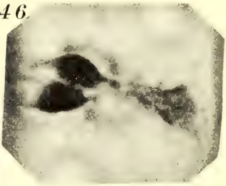
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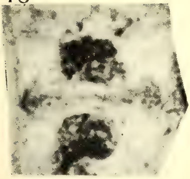
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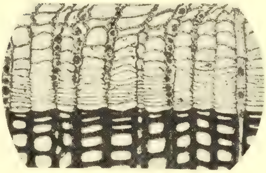
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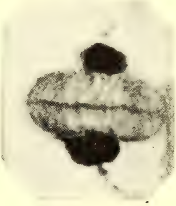
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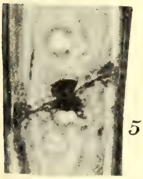
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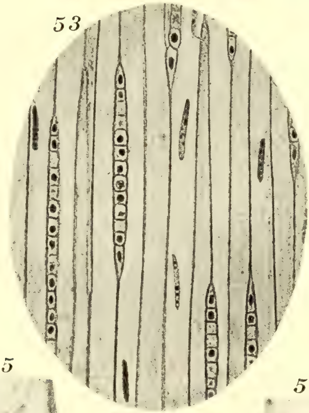
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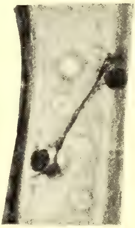
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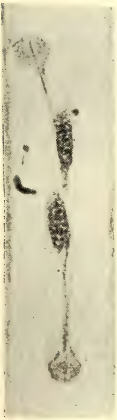
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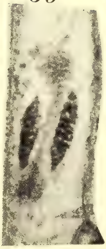
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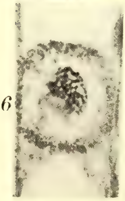
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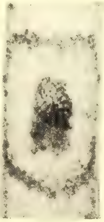
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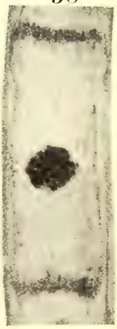
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BAILEY: CYTOLOGICAL PHEMOMENA IN CAMBIUM.

MORPHOLOGY AND LIFE HISTORY OF SOME ASCOMYCETES WITH SPECIAL REFERENCE TO THE PRESENCE AND FUNCTION OF SPERMATIA[1]

B. B. Higgins

The presence of "spermatia," of "microspores," or of a "Phoma" or "Phyllosticta" stage has been mentioned in connection with numerous species in various families of the Ascomycetes; but usually the function or the genetic connection has not been studied, consequently our knowledge of these structures is still very hazy. Do they occur regularly at a definite stage in the development of the fungus, or only under exceptional conditions? Do they function, like ordinary spores, as reproductive bodies for propagating the fungus; or are they sexual elements? If the latter, are they now functional or merely degenerate remnants?

These are questions which have been determined for only a few species outside the Laboulbeniales and the lichens. The spermatia of *Gnomonia erythrostoma* were described by Frank (4) and those of *Polystigma rubrum* by Fisch (3); and in both cases they were thought to be male sexual elements. This view has been sustained by the later studies of Brooks (2), and Blackman and Welsford (1), who considered them to be male sexual elements now functionless through degeneration of the carpogonia. Nienburg (8) considered *P. rubrum* to be a true oogoniate with the spermatia as degenerate functionless elements. Müller (7) found that the spermatia of *Rhytisma acerinum* failed to produce infection on the host plant or to germinate in culture media; and, failing to find carpogonia, he concluded that the spermatia were functionless male elements. The writer has reported the occurrence of spermatia and carpogonia in connection with the young ascocarps of three species of *Coccomyces* (5) and of *Mycosphaerella nigerristigma* (6). In these forms also the spermatia failed to germinate in any culture medium tried.

Further observations have shown that similar structures occur quite commonly in many families of Ascomycetes; and the question as to their possible function has led to a study of the complete life history of several species, some of which have not been previously described. The observations have been confined very largely to the species growing parasitically on the leaves and succulent parts of flowering plants, because of the comparative ease with which such forms may be studied and of the improbability of the association of other species, and also because of the value to plant pathologists of knowing the complete life history of such parasites.

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[1] Paper number 14, Journal Series, Georgia Agricultural Experiment Station.

Besides the studies on the life history of these forms, it seems best to report at this time certain observations on the occurrence of spermatia and the development of the ascocarps in several other species. Because of the desirability of recording all observations on the details of morphological development and on parasitism, each species will be considered independently, before taking up a general discussion of the occurrence and function of spermatia.

Sphaerella Bolleana n. sp.

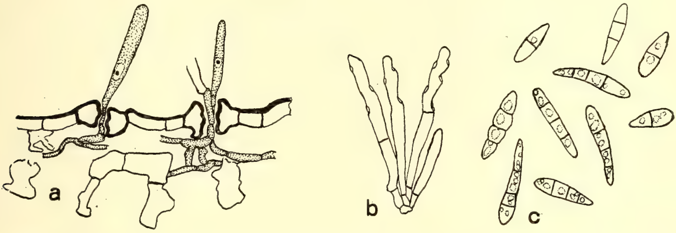
Cercospora Bolleana (Thum.) Speg. is very common and widespread as a parasite on the leaves of the fig, *Ficus carica* L., producing small, irregular brown spots two to five millimeters broad. When infection becomes very abundant distinct spots are not formed, but the whole under surface of the leaf becomes covered with the dark olive-brown conidiophores and the leaf soon drops from the tree.

The development of the spots is very slow and irregular; and this, with the fact that conidiophores may often be found on the lower surface before a distinct spot is discernible above, suggested that the leaf tissue is killed, not by the direct action of the fungus but by the drying of the tissues through the epidermis broken by the emerging conidiophores. In view of the fact that most parasitic species of this genus kill the host tissue rapidly, apparently by means of some poison secreted by the fungous mycelium, some study was made of the development of the spots and of the relation of the mycelium to the leaf tissue.

The results from many artificial inoculations show that about a month is required for a typical leaf spot to form. Infection seems to take place through the stomata, since placing the spores on the upper surface of the leaf failed to produce infection; though the actual entrance of the germ tube has not been observed. The first sign of disease is a browning of the epidermal cells at the point of infection, which begins to appear in from five to ten days. By the end of two to three weeks, tiny brown spots also begin to appear on the upper surface. These gradually enlarge and coalesce until a fully developed spot is formed.

Microscopical examination of sectioned and stained material showed the reason for this peculiar feature. The mycelium grows very slowly and is mostly intracellular. The host cells actually penetrated are killed; but, for a time, the adjoining cells seem to suffer very little injury. Evidently some poisonous substance is produced; but it is of such a nature that it does not diffuse rapidly from cell to cell. Very often the hyphae enter the vascular bundles and spread much more rapidly, sending out branches through the pits in the vessels and killing the surrounding cells. The hyphae are slightly constricted on passing through a cell wall, and do not disintegrate the walls to any appreciable extent.

The conidiophores arise as simple branches which are pushed out through the stomata (text fig. 1, *a*). Later branches arise from the basal cell of the older conidiophore within the stoma, often forming a fascicle of six or eight conidiophores of varying ages. The fascicle may often be scraped off and remain fastened together by this basal cell.



TEXT FIG. 1. Conidiophores and conidia of *Sphaerella Bolleana* from fig. leaf: *a*, section of lower portion of leaf with young conidiophores emerging through stomata; *b*, clump of old conidiophores; *c*, conidia, showing variations in size and shape. *a*, × 580; *b* and *c*, × 300.

Artificial Cultures. On culture media the growth of the mycelium was also very slow. Cultures were obtained by planting the conidia on agar plates and then transferring the conidia, after germination, to plates of sterile agar or to various other media. The germ tubes branch very profusely, forming a small dense colony. The mycelium is at first colorless but after four or five days begins to darken and gradually changes to various shades of olive depending upon the nature of the substratum.

On bean agar and on steamed green bean pods the colonies are small (usually less than a centimeter in diameter), circular in outline, and slightly raised. The base of the colony is slightly stromatic, and composed of black, thick-walled cells. This is covered with a velvety growth of gray to olive-brown hyphae.

On steamed Irish potato plugs the growth is less vigorous than on bean pods; though otherwise it is very similar. Apparently the fungus is not able to assimilate the potato starch.

Steamed sweet potato plugs gave the best growth of any medium tried. The growth was more rapid and the ultimate size of the colony much greater than on the other media. At the end of six weeks the colonies had, in most cases, practically covered the plugs which were one and a half centimeters in diameter by about four centimeters in length. The colony was capitate, raised half a centimeter or more at the center. The base, next the substratum, was composed of large, black filaments which broke up easily into individual cells. The surface growth was composed of more slender hyphae light gray in color with a tinge of pink toward the center of the colony.

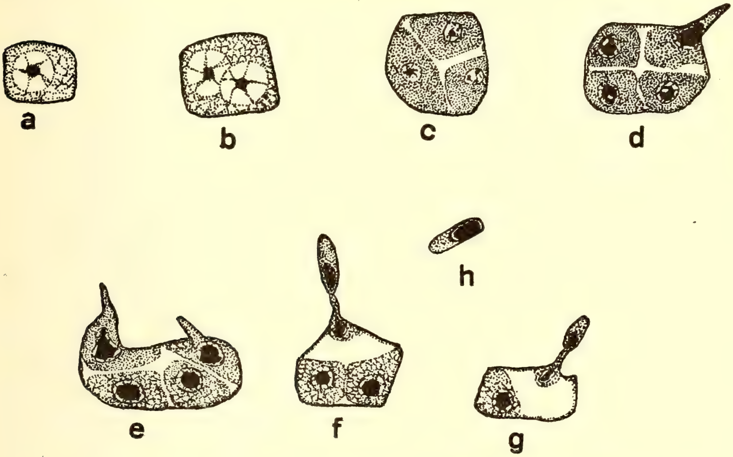
No spores of any kind were ever produced in cultures; though a great many media, including those mentioned above, steamed fig leaves, steamed filter paper, steamed corn meal mush, steamed corn grains were tried.

Spermatia. The first spots usually appear on the fig leaves during the month of June; but, because of the slow growth of the fungus, infection is generally not sufficiently abundant to cause leaf casting until the latter part of August. About the time the diseased leaves fall, the fungus shows remarkable activity. Pycnidium-like structures develop very abundantly. These are the spermogonia. The first indication of their development is a profuse branching and coiling of the hyphae at the base of the old conidiophores or at points where new conidiophores are being pushed out (see figs. 2 and 3, Pl. XXX). Such new conidiophores seem to develop abnormally and do not produce conidia. The weft of intertwining hyphae develops into a globose to oval mass which soon breaks the leaf epidermis and pushes out beyond. The cells of the mass enlarge. Those toward the surface coalesce to form the wall of the spermogonium, while toward the center the spermatiferous cells become richer in protoplasm and begin the process of forming spermatia even before the wall of the spermogonium is formed (fig. 4).

In this process the cell enlarges slowly, while the nucleus grows much more rapidly and soon fills almost the entire cell cavity (text fig. 2, *a*). The nucleoplasm stains very faintly, and the nucleolus at this stage is comparatively small. The cytoplasm is granular or finely alveolar in structure. The nucleus now divides, but no cell division occurs at this time. The two daughter nuclei reorganize rapidly, soon reach approximately the size of the original mother nucleus (text fig. 2, *b*), and both divide again. After this second division the four nuclei remain rather small, and the nucleoli do not reorganize at once (text fig. 2, *c*). Apparently chromatic material is scattered throughout the cytoplasm, which is now very difficult to destain. Following this second nuclear division, the cell divides (by cleavage) into four approximately equal parts without formation of separating walls. Each of the four nuclei with its surrounding cytoplasm ultimately forms a spermatium; and these four daughter cells may, therefore, be termed young spermatia while remaining within the mother cell wall. One or more sterigmata are now pushed out from each spermatial mother cell (text fig. 2, *d*); and the young spermatia pass out, one at a time, each forming a mature spermatium at the apex of the sterigma (text fig. 2, *e–h*).

After the young spermatia have passed out of a mother cell wall, some slightly stainable substance remains. This seems to be a mucilaginous or gelatinous substance that was formed instead of a wall around the young spermatia. It probably plays an important part in creating pressure to force the young spermatia out of the mother cell wall, and also in carrying the mature spermatia out of the spermogonium. The mature spermatia are carried out of the spermogonium and held in a mass at its apex (fig. 5, Pl. XXX) by some such substance; and, when wet, they spread out over the surface of the leaf.

Spermatia continue to develop until about the middle of December. At this time the spermogonium is a globose or conical structure with a very thin wall composed of two or three cell layers. There is a small pore, but no beak, at the apex. The cells which make up the wall of the spermogonium are thick-walled and brown, and are readily differentiated from the thin-walled and colorless spermatiferous cells.



TEXT FIG. 2. Stages in the development of the spermatiferous cells and the spermatia: *a*, spermatiferous cell just before the first nuclear division; *b*, just before the second nuclear division; *c*, just after the formation of the young spermatia, the fourth spermatium being beneath the three shown; *d*, single sterigma and four young spermatia showing within the mother cell wall; *e*, two sterigmata formed by one mother cell; *f*, *g*, spermatia passing through sterigmata; *h*, mature spermatium, showing large nucleus almost filled by the nucleolus. All drawn with the aid of a camera lucida. × 4200.

Perithecia. The perithecia begin their development coincidentally with that of the spermogonia, or perhaps a few days later; and in the early stages it is not possible to say which are spermogonial and which perithecial primordia. Very soon, however, they may be differentiated by means of the changes which occur in the cells that make up the weft. In the young spermogonium the spermatiferous cells begin to develop; while in the center of the young perithecium the carpogonium, a single filament, becomes very conspicuous in stained material because of its great affinity for protoplasmic stains. Occasionally two carpogonia develop in a single perithecium.

The carpogonium arises from the base of the young perithecium; and the free end extends up to and often beyond the apex of the mass of hyphae, which by this time has broken through the leaf epidermis (figs. 6, 7). It is very much enlarged and coiled, usually making one complete turn, at the base; but it tapers gradually toward the free end into a very slender trichogyne. The trichogyne is generally erect, extending directly toward the apex of the young perithecium except where it is bent by pressure of the other hyphae. There is no distinct line of demarcation between the enlarged basal portion and the trichogyne. As the terms are used here, the "basal portion" includes only the part that is coiled, and all the erect portion is the "trichogyne." There are two comparatively large nuclei in the basal portion and two much smaller in the trichogyne (fig. 6). In some cases these nuclei are separated by cross walls; but the entire structure, at this stage, takes the stain so evenly that it is extremely difficult to determine details of structure, and it is not possible to say at what stage the cross walls are formed. Observations indicate that they are laid down only after disintegration of the protoplasm has begun at the tip of the trichogyne.

The protoplasm of the trichogyne soon becomes granular and disintegrates, this process beginning at the tip. This continues until only about half the coiled basal portion remains. At this stage two healthy appearing nuclei remain lying close together in the remainder of the basal portion. This cell becomes the ascogonium and later on gives rise to the ascogenous hyphae.

By the time disintegration of the trichogyne is completed, the cells of the surrounding hyphae composing the young perithecium have coalesced to form an almost solid mass of pseudoparenchyma; but the perithecium continues to enlarge, apparently by formation of new cells over the surface. The perithecium soon reaches its full size. The outer cells develop thick, brown cell walls and become the permanent wall of the perithecium. The cells in the center remain thin-walled and colorless and are broken down by the later development of the asci (figs. 8, 9).

Both the question as to the origin of the two nuclei in the ascogonium and the question as to their fusion must remain unsettled for the present.

After a short rest the ascogonium becomes rapidly multinucleate. The nuclei are paired in the ascogonium and pass out in pairs into the ascogenous hyphae (fig. 8). Here they divide, one pair of the four resulting nuclei in each ascogenous hypha passing to the tip of the hypha, the other pair remaining in the base. A cross wall is now formed separating the two pairs of nuclei. The terminal cell enlarges and becomes the ascus. The two nuclei in the ascus soon fuse. The ascogenous hyphae apparently do not branch; and there is no crozier formation. Nearly all the cytoplasm of the ascogonium passes with the nuclei into the ascogenous hyphae, leaving the ascogonium almost empty and very difficult to distinguish; but in some cases its wall may yet be seen when the asci are nearly mature.

This stage in the development of the young asci is reached early in the winter, before the end of December. They then seem to pass through a necessary resting stage. All attempts to hasten the maturity of the perithecia and the formation of ascospores by placing the leaves bearing them in a moist chamber in the laboratory at this period have resulted in failure; although, after about the first of February, mature ascospores may be obtained by this same method in from one to four days.

The nuclear divisions in the ascus have not been studied carefully. They seem to occur at irregular intervals during the spring and winter. This irregularity, together with the difficulty of proper killing and fixing of material, makes such a study very tedious. The colorless pseudoparenchyma in the interior of the perithecium is of such a nature as to prevent penetration of Flemming's solution and other killing agents of the chromic acid group, which give very good results at all other stages. The only solution which has proved at all satisfactory is one made by dissolving $1\frac{1}{2}$ grams of picric acid in 100 cc. of 70 percent alcohol and then conducting into this solution the fumes from 1 gram of $NaSO_3$ treated with a few cubic centimeters of sulphuric acid. After these colorless cells had been crushed by the growth of the asci, that is, when the asci were nearly mature, Flemming's solution gave excellent preparations. Asci containing four nuclei have been found during January, and by the first of March the young ascospores are beginning to form; but the spores do not mature until the early part of May.

The spores are imperfectly biseriate in the ascus. They are at first continuous; but when mature they are septate, each spore being constricted into two slightly unequal cells. The smaller cell, toward the base of the ascus, is pointed at the end; the other cell is thicker and rounded at the apex. The ascus is slightly thickened at the apex but does not open by a pore.

The maturity of the perithecia is remarkably uniform. Spore discharge continues during only a few days, if the leaves remain damp.

The ascospores are apparently the only means by which the fungus is carried through the winter and are probably responsible for all spring infection of the host plant. No conidia of any sort have ever been found during the winter and spring. The conidiophores appear to be dead soon after the leaves fall. The first spots begin to appear on the older leaves of the host about a month after the ascospores mature.

Genetic Relationship of the Spore Forms. The relation of the spermogonia and of the perithecia to the Cercospora stage is shown by their development at the base of, and direct connection with, the conidiophores. Very often remnants of these old conidiophores may be seen on the perithecial walls when the ascospores are mature in the spring. Frequently also a spermogonium and a perithecium develop side by side with a single wall between the two cavities (see fig. 8). This observed connection was also corroborated for the conidial and ascigerous stages by comparison in cultures and by inoculations. All attempts to germinate the spermatia failed.

Cultures obtained from single ascospores were grown on steamed green bean pods, bean agar, steamed Irish potato plugs, steamed sweet potato plugs, and corn meal mush, in comparison with cultures obtained from the Cercospora conidia. The resultant colonies were similar in every particular of shape, size, coloration, and general development, and neither produced spores of any sort.

The facts that no spores were obtained in cultures and that the old mycelium from cultures failed to produce infection made inoculation with pure cultures very difficult. Infection with the production of spots and Cercospora spores was obtained several times by crushing perithecia, containing mature ascospores, in a drop of water which was placed on the under surfaces of fig leaves in the greenhouse, the plants being kept under bell jars for a few days; but one could not be positive with this method that some conidia had not been included with the perithecia. It was therefore necessary to devise some method for inoculating with ascospores of known purity. For this purpose the idea of isolating in agar single spores or single asci containing germinating spores was hit upon.

On March 16, 1916, perithecia containing mature ascospores were crushed and plated out in agar. The next day ascospores and asci containing spores were located with a microscope and their position was marked. The spore or ascus, with a surrounding block of agar, was then lifted out with a sterile scalpel and transferred to the surface of sterile agar in another plate. These transferred blocks were examined under the microscope at intervals for two days, and only those blocks which showed no contaminating organism were used in making inoculations. Eight such blocks were then transferred to the under surfaces of leaves of fig plants grown in the greenhouse from potted roots. Two typical spots resulted, and at the end of two months Cercospora spores were abundant on these spots. The experiment was repeated on April 10, and four spots developed in a similar manner.

While the percentage of infection was small, there can be little doubt that the infections were produced by the ascospores, since there was no possibility of Cercospora spores being present on the leaves and the checks receiving blocks of sterile agar developed no spots at all. The agar probably held the spores so far from the leaf surface that the germ tubes had in many cases lost their power of entering the tissue before reaching the leaf surface. Abundant infection always resulted when the ascospores were placed directly on the leaf surface.

Systematic. The structure of the perithecia, together with the eight-spored asci lacking the apical pore and the two-celled, hyaline spores, places the fungus unquestionably in the genus *Sphaerella* Ces. et de Not. So far as the writer has been able to find, no similar *Ascomycete* has been described as occurring on the leaves of the fig; and since—from the nature and sequence of development of the stages of the fungus—it is not likely to occur except on leaves parasitized by the conidial or *Cercospora* stage, describing it as a new species seems justified.

In order to facilitate the association of the new name with the well known disease of the fig, the specific name, *Bolleana*, applied to the conidial stage is used; and the name *Sphaerella Bolleana* is suggested with the following diagnosis.

Mycosphaerella Bolleana[2] n. sp. Perithecia mostly hypophyllous, partly embedded in the leaf tissue, erumpent, 60–105 × 55–95 μ, black; ostiolum papillate; asci cylindrical to club-shaped, almost sessile, eight-spored; spores club-shaped to cylindrical, two-celled, 17–20 × 3.5–5.5 μ, hyaline.

Spermogonia produced in the autumn, hypophyllous, embedded in the leaf tissue, with only the ostiolum emergent, ovate, 40–90 × 30–75 μ; spermatia small, rod-shaped, 2–3 × 1 μ, hyaline.

Conidial stage: Spots ferrugineous to olive-brown, irregular, 2–5 mm. in diameter; conidiophores hypophyllous, simple, solitary or fasciculate, slightly geniculate, brownish olive, continuous or sparingly septate, 50–90 × 5–6 μ; conidia olive-brown, clavate to fusoid, 32–53 × 6–8 μ; 1- – 5-septate.

Conidial stage parasitic on the leaves of *Ficus carica* L.

Perithecia and spermogonia on the dead fallen leaves.

Peritheciis hypophyllis, semiimersis, sparsis, ovatis, 60–105 × 55–95 μ; ostiolis prominulis; ascis cylindricis vel clavatis, brevissime stipitatis, aparaphysitis, 35–40 × 11 μ, octosporis; sporidiis hyalinis, clavatis, 17–20 × 3.5–5.5 μ, 1-septatis.

Spermogoniis autumno, hypophyllis, immersis, emergentibus, punctiformibus, nigris, ovatis, 40–90 × 30–70 μ; spermatiis minutis, cylindricis, 2–3 × 1 μ, hyalinis.

Hab. in foliis dejectis *Fici caricae.*

Status conidicus: Maculis brunneis vel olivaceo-fuscis, irregularibus, 2–5 mm. lat., interdum subeffusis; hyphis hypophyllis, solitariis aut fasciculatis, apice geniculatis, continuis aut septatis, 50–90 × 5–6 μ; conidiis clavatis vel tereti-fusoideis, 32–53 × 6–8 μ, apice obtusioribus, chlorino-olivaceis, 1- – 5-septatis.

Hab. in foliis vivis *Fici caricae.*

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[2] The technical description bears the generic name Mycosphaerella Johanson, since this name is used in many recent systematic works; though it seems desirable that Sphaerella Ces. et de Not. may be one of the *genera conservanda* in the report of the committee appointed by the Botanical Congress at Vienna in 1910.

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EXPLANATION OF PLATE XXX

(All figures × 580)

FIG. 2. A young spermogonium (or perithecium) in the lower side of a fig leaf, developing at the base of conidiophores; from material killed October 16, 1915.

FIG. 3. Young spermogonium (or perithecium) breaking the host epidermis.

FIG. 4. Young spermogonium in which spermatia are beginning to form. The wall not yet differentiated.

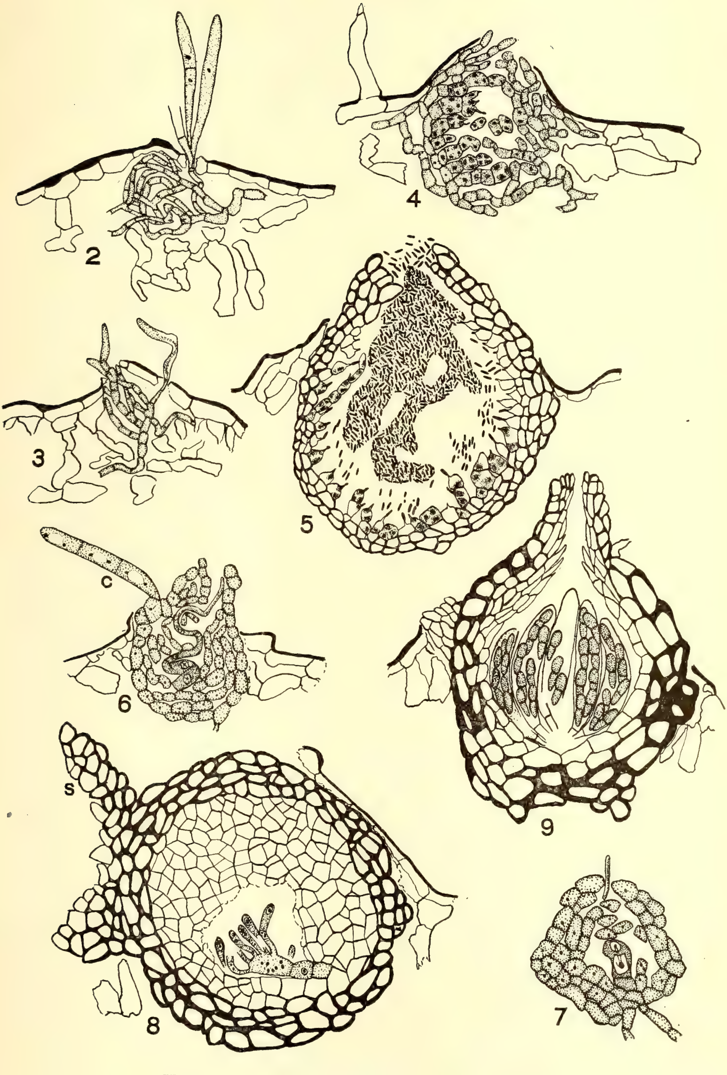
FIG. 5. Mature spermogonium with spermatia being pushed out in a mucilaginous mass.

FIG. 6. Young perithecium with four-nucleate carpogonium. *c*, an abnormal conidiophore.

FIG. 7. Young perithecium with binucleate ascogonium; portion of dead trichogyne still persistent at the apex.

FIG. 8. Slightly excentric section of perithecium, with ascogenous hyphae and young asci. *s*, portion of the wall of an old, empty spermagonium.

FIG. 9. Section of mature perithecium.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HIGGINS: FUNCTION OF SPERMATIA IN ASCOMYCETES.

BIOLOGY, MORPHOLOGY, AND CYTOPLASMIC STRUCTURE OF ALEURODISCUS

HARVEY E. STORK

BIOLOGY OF ALEURODISCUS AMORPHUS

The results here reported are the outcome of observation and succeeding laboratory study of *Aleurodiscus amorphus* (Pers.) Rabenhorst as found on the balsam fir in the Adirondack Mountains, vicinity of Seventh Lake, Hamilton County, N. Y. This plant has been described under a number of different names, a fact due, in part, to its pezizoid form. Persoon first described it as *Peziza amorpha.* Fries made it a Thelephora and later a Corticium. Quelet put it into the genus Cyphella, and more recently Peck described plants from the Adirondack region as the type of a new genus Nodularia. The plant has characteristic moniliform paraphyses that give a suggestion of an ascus with large spores when examined under low magnification, and this has been one of the misleading characters. In dried plants, too, the contents of the basidia often round up into globular masses which have been mistaken for ascospores.

This Aleurodiscus bears the distinction of being the first Basidiomycete in which a nucleus was definitely described, De Bary (7) having reported and figured in 1866 the fusion nucleus in the basidium. It is not surprising that this should have come to his attention, for the mature basidia are very large, reaching the size of 150 × 24 μ; and the fusion nucleus (reaching 15 μ) is the largest that the writer has seen in the fungi or of which he finds report in the literature, if the peculiar nucleus of the chytrids be excepted. It was for this reason that the late Professor G. F. Atkinson suggested this plant to the writer in 1915 as a good object for cytological study. Because of the large size of the nuclei, especially in the large basidia, it has been found a most favorable object for the study of nuclear fusion and of the behavior of the elements of the nucleus in karyokinesis. The study of this phase of the subject is, however, incomplete in some of the stages, and a discussion of the details of karyokinesis is left for a subsequent paper, the cytological part of the present study being limited to cytoplasmic structures.

Another species of Aleurodiscus that resembles in many respects the one here discussed is *A. Oakesii*, commonly found on the bark of living Ostrya and less frequently on the bark of several other frondose trees. This species is also being studied by way of comparison with *A. amorphus.* Their fruit bodies are somewhat similar, but they are never found on the same hosts. The fruit bodies of *A. Oakesii* (upper and middle figures, Plate XXXI) are normally more or less cup-shaped, while those of *A.*

amorphus (lower figure, Plate XXXI) are normally convex. Those of the former species also tend to be larger and more subject to confluence. Fries (8) considered the two species identical, and more recently Morgan (18) made *A. Oakesii* the same as *A. amorphus*. The character of the paraphyses of the two is, however, quite different, those of *A. amorphus* being moniliform and those of the other species of a peculiar bottle-brush type. These differences were pointed out by Cooke (4) and later by Peirce (19).

The fruit bodies of *A. amorphus* develop during the summer on the surface of twigs and small branches of fallen *Abies balsamea*. They have never been observed on branches larger than three centimeters in diameter. They do not occur on the twigs and branches of living trees, nor on those of trees that have been dead for too long a time. The fungus seems to be definitely selective in the degree of decay of its substratum. During the observation of each of the four summers, the numerous balsam firs that had been blown down by the wind since the preceding summer were never seen to harbor the fungus. But during the second summer after the falling of the tree, the fungus was seen to produce its orange-yellow fruit bodies abundantly, and they might recur in the third summer, but in the fourth summer in the history of a fallen tree no evidence of the life of the fungus was ever observed. In two relatively low fern swamps kept under observation, in which there were numerous balsam firs, some of which were uprooted by the wind each year, one could predict almost with certainty upon which of the fallen trees *A. amorphus* could be found during any one summer. Aside from the balsam fir several other conifers are reported as harboring the fungus as a bark saprophyte, *viz.*, *Abies concolor*, *Thuja plicata*, *Picea* sp., *Tsuga* sp.

The fruit bodies usually are very abundant on the lower and moister sides of twigs and branches and are seldom seen on the upper sides unless these are very well protected from dessication. The habit photographs (Plate XXXI) were made by pointing the camera upward under fallen trees. In descriptions of this species as well as of others of the genus the statement is often made that the fruit bodies are incrusted with mineral matter to such a degree as to make structural studies difficult. In all material used by the writer no incrustations were ever observed, and mineral crystals among the hyphae have never interfered with the making of sections.

A description of the species will not be repeated here. The reader is referred to the description given by Burt (3). A feature not noted in any descriptions is that the mature spores in mass present a distinctly pink tinge, although by transmitted light the spore coat appears hyaline. Hennings (11) describes the color of the hymenium as being at first scarlet, then becoming paler. No material has ever been collected by the writer that had any suggestion of red. The fruit bodies are at first of a yellowish-orange color that may later become paler and even a light buff. The plant no doubt varies in different localities in some of its characters, as is also indicated by Burt's report that, in collections from Idaho westward, the echinulate marking of the spores is very faint.

Another saprophyte of the balsam tree that in the form of its fruit body bears a superficial resemblance to Aleurodiscus is *Dasyscypha Agassizii* (Berk. & Curt.) Sacc. The two are often found side by side, and they are the first two conspicuous saprophytes that attack the tree. After two or three years, when the bark of the twigs becomes somewhat loose from decay, numerous other saprophytes are encountered, most common of which are *Polystictus hirsutus* (Wulf.) Fr., *P. pergamenus* Fr., *Lenzites betulina* (L.) Fr., *Panus stypticus* (Bull.) Fr., *Creonectria cucurbitula* (Sacc.) Seaver, and *Poria* sp.

Aside from the plants above mentioned, the writer has very frequently encountered a small species of Tremella that grows upon the fruit bodies of the Aleurodiscus. Indeed, in more than half the cases in which the latter were found on fallen balsam trees, the Tremella was present. It appears in the form of hyaline glistening droplets on the fruit bodies of the Aleurodiscus, varying in size from microscopic bodies to forms that completely cover the surface of the plant. Cross sections of such fruit bodies harboring the Tremella are shown in figures 9 to 11, Plate XXXII. So far as the writer knows, the plant is truly parasitic and is confined to this host. It was never seen growing on the bark of the balsam fir away from the Aleurodiscus, nor on the other fungi mentioned above as commonly encountered by the side of this one. The Tremella hyphae grow down into the hymenium and subhymenial tissue of the Aleurodiscus and mingle intimately with those of the latter. Beneath the parasite the hyphae of the Aleurodiscus soon cease active growth and for a long time remain in a degenerating condition before they are killed. It is in this condition that the nucleus shows characteristic structures and the cytoplasmic granules undergo significant changes that are to be discussed in detail later.

The writer is unable to identify the Tremella with any species previously described. The species that perhaps approaches it most nearly is *Tremella versicolor* Berk., which was described in Europe as "parasitic on *Corticium nudum* on decorticated trees" (Berkeley, 2). This plant, however, is described as orange in color, at length assuming a rufous tinge, while the one here in question is always hyaline, never assuming even the color of its host. The fruit body consists of an interwoven mass of much branched slender hyphae ($2\frac{1}{2}\ \mu$) which secrete a viscid material that gives the whole a gelatinous consistency. When young, and even at the very beginning of its growth, its surface is usually covered with abundantly branched conidiophores. The conidia are elliptical in shape, $5 \times 7\ \mu$, and stain very densely with safranin or haematoxylin. Large numbers of them are often seen embedded within the lower parts of the older tissue. A very definite hymenium is formed of the globose basidia that divide in the cruciate manner characteristic of the Tremellales. This hymenium stands out in the photomicrograph (fig. 10), the black dots representing basidia. As the sterigmata grow from the four cells of a basidium, the cells split apart to some extent downward. The globose basidia measure 15 μ in diameter when mature, and the sterigmata are slender like the hyphae of the plant and attain a length (up to 30 μ) that gives them the general appearance of germ tubes rather than of sterigmata. It was suggested that the Tremella be described as a new species, but the characteristics that are really distinguishing are so few in this group that it is perhaps better not to multiply species until the group is better understood.

Several efforts were made to infect the fruit bodies of *Aleurodiscus Oakesii* with the Tremella by introducing the mycelium and the conidia on the young fruit bodies. The Tremella never showed any active growth when thus transferred.

After the Tremella has had opportunity to grow for some time upon the fruit bodies of the host, these latter are often so completely incrusted that one fails to recognize their identity, and the twig appears to be covered merely with a group of little pulvinate plants of the Tremella. Two such fruit bodies are shown in cross section (figs. 10, 11). This leads us to recall other cases of Tremellas associated with different fungi. In 1894 Dangeard (5) described and figured the association of the mycelia of *Dacryomyces deliquescens* and *Tremella* sp., the two finally forming a common hymenium. He speaks of this as a case of symbiosis. Fries (8) described the species *Tremella biparasitica* growing on the stipe of *Nyctalis parasitica*, and *Tremella parasitica*, reported by Schweinitz as growing commonly on *Clavaria gigantea* Schw. in North Carolina. Of this, he uses the significant phrase, "non a Clavaria separabilis."

It is easy to think of the plants commonly placed in the genus Tremellodendron as members of the Thelephoraceae that have Tremellas intimately associated with them. The characters of the hymenium most closely resemble those of the hymenium of Sebacina Tul., which is a genus with decidedly incrusting tendencies. Other genera in this class are Protohydnum and Protomerulius of A. Möller.

However, regardless of what our surmises may be concerning the composite nature of the plants of these genera, we can arrive at definite proof only by producing them through "synthesis" in cultures. In this connection I quote from a personal letter of Professor R. A. Harper:

"There are a number of forms which have been listed under various other genera which are, in my opinion, incrusting if not parasitic members of the Tremellineae. I have always had a suspicion that the Tremellodendron question is mixed up with something of this sort."

Development and Morphology of the Fruit Body

Pure cultures of the mycelium of both *Aleurodiscus amorphus* and *A. Oakesii* have been obtained by suspending the fruit bodies in test tubes of nutrient agar for a time and allowing the spores to fall upon the agar. The mycelium of *A. amorphus* has never produced fruit bodies in these cultures. Its spores germinate only occasionally in water, and the various decoctions used, including those of Abies bark, have been ineffective for inducing germination. The complete life cycle of the plant has therefore not been traced, nor is it known how infection of the fallen Abies trees takes place. The mycelium of the fungus is found growing through the bark tissues and the cambium. The mycelium remains intercellular. It never enters the phellogenous tissues, and when the other elements of the bark are disintegrated the corky layer remains intact. It is evident as a lightly stained layer, several cells deep, just beneath the epidermis of the bark in figures 1 to 7. In certain areas the mycelium concentrates and forms a cushion-like stroma of pseudoparenchymatous tissue in the bark parenchyma. The hyphae are thicker here than are those that ramify among the elements of the bark, and they branch frequently. As this stroma grows, the surface of the bark is arched upward to form a superficial prominence (figs. 1, 2). There now sets in a strong upward growth of the upper stromal hyphae and the pressure produces a rupture in the bark by which the rapidly elongating hyphae emerge (fig. 2). They branch frequently and continue growth until they attain the form represented in figures 3–5.

The hymenium now begins its formation in the even upper surface. In figure 6 the hymenium is shown in the process of development. The marginal hyphae are longer and more slender from the first, so that under a hand lens they present the appearance of a white hairy margin. Occasionally, in some fruit bodies, these hyphae grow much more rapidly than do the central elements of the fruit body, with the result that the structure is decidedly concave, instead of convex as is typically the case (fig. 7). In some cases, the marginal hyphae have grown so rapidly as to arch over almost completely the young hymenium, so that in sections of these fruit bodies made somewhat tangentially they appear altogether gymnocarp because of the perithecioid form of the structure. This latter method of development the writer attributes to dry conditions of growth, as it is usually encountered on the drier branches of the firs.

In the hymenium the most conspicuous elements are the large basidia with their prominent fusion nuclei. Beside them are the nodulose or moniliform paraphyses and the more slender filiform paraphyses. The basidia keep pace uniformly in their growth so as to present an even, level peripheral surface. The paraphyses project usually about 25 μ above the general level. When a basidium begins putting out sterigmata it elongates so as to stand some 25 μ above the general level of the younger basidia. These points are brought out in the photomicrograph (fig. 12), in which one old basidium with sterigmata is shown elongated so as to attain the general level of the paraphyses. In thin sections only two of the four sterigmata are usually seen. To some extent, the age of a basidium can be ascertained from this elevation above the surface. Another check on the degree of advancement of the nuclear divisions is the length of the sterigmata and the size of the primordial spore vesicles. The two divisions of the fusion nucleus are completed when the sterigmata begin to form (fig. 23, Pl. XXXIII). The nest of four daughter nuclei rests for a relatively long time while the sterigmata are elongating and the spores are developing.

The young hyphae and the paraphyses in the hymenium are always binucleate (figs. 17, 18). The nuclei in the primordial basidial cell fuse quite early, even before the basidia have begun to enlarge to any great extent. The fusion nucleus can be easily recognized by its relatively large size, its elongated shape, and especially by the character of the chromatin material (figs. 19, 20). This remains in the form of a spireme throughout the period of the growth of the nucleus until the first nuclear division. It seems that there are many more basidia formed than ever come to sporulation. The upper part of the hymenium is crowded as a result of the large size of the sporulating basidia, and many smaller ones are left below, lacking room for further development.

Cytoplasmic Structure

The cytoplasm of the hyphae and especially of the basidia presents some very striking appearances when fixed in Flemming's medium solution and stained with haematoxylin. In the young, actively growing hyphae and in the basidia, it consists of a finely granular ground substance in which are embedded larger elements. These larger elements occur in two forms. The most abundant form is that of round or somewhat elongated corpuscles varying in size from minute granules to relatively large bodies reaching 1 μ in diameter. Less abundant is the second form, which consists of long filaments that may attain a length of 40 μ or more. Both types of structures present the same appearance under different kinds of staining, and an examination of the preparations leads one to conclude that they are the same kind of substance differing only in form.

The corpuscular forms appear most strikingly in the basidia, for here they are more abundant and often of larger size than in the other elements. They are not distributed uniformly throughout the cytoplasm. The tendency is for them to be most numerous in the upper part of the basidia while in the lower part of the basidia they are usually of somewhat larger size though fewer in number. In many cases they are aggregated about the fusion nucleus of the basidium so as to obscure the detail of the nuclear structures, and this aggregation is usually seen in the upper part of the nucleus so as to give the appearance of a sort of cap (fig. 21). In this connection we are reminded of the description and figures presented by Janssens *et al.* (12) of the mitochondria in the young ascus. They speak of them as forming a cap on one side of the nucleus. In a number of other features the mitochondria described by Janssens agree with the cytoplasmic structures here described. Guilliermond (10), in the first report of the occurrence of mitochondria in fungous tissues, had also described and figured a perinuclear aggregation of these structures in the ascus of Pustularia.

At times the area of more densely aggregated granules is situated higher than the nucleus in the basidium or appears to be moving upward toward the apex of the basidium (fig. 23).

When the basidium puts out sterigmata and forms spores, the granules appear in the cytoplasm of these structures (figs. 23, 28). In fact, they make it difficult to follow the nuclear phenomena here. In the spore at the left in figure 28, for instance, it is difficult to tell whether the spindle-like structure is a nuclear spindle or a group of the corpuscles gathered about the middle of a filament.

The second type of structure, the filamentous form, occurs in both old and young basidia and spores and is occasionally seen in young growing hyphae and paraphyses. These filaments vary considerably in length and thickness, at times attaining a size so large as to appear like a foreign body thrust into the basidium. They are generally straight but may be curved, wavy, or slightly spiral. Occasionally two are seen lying parallel and closely approximated. They almost invariably extend in the direction of the long axes of the hyphae or basidia. The position occupied by one of the three shown in figure 22 is exceptional. Almost every basidium contains from one to four of these filaments. It is seldom that as many as six are encountered in the same basidium.

The filaments are relatively strong, rigid structures. This is easily ascertained by a study of sections that were somewhat broken up in the course of preparation. Here the filaments are often seen projecting out of the end of a broken basidium and maintaining themselves in a rigid position. Several cases, too, were encountered in which a sterigma was broken loose and yet seemed held in place by the unbroken filament (fig. 24).

There is a general tendency for the filaments to be directed toward the apex of the basidium, and when the sterigmata form, the filaments frequently extend into these structures. In fact it is quite common to see a filament extending from the basidium into the sterigma up to its apex.

When the amount of acetic acid in the Flemming's solution is decreased the corpuscular bodies appear even more numerous, although they stand out less definitely than when Flemming's solution is used. This virtually gives Benda's solution used so extensively for mitochondrial fixation. Likewise, Hermann's fluid gives equally good results. With Carnoy's and Zenker's fixatives the cytoplasm presents no structures in the finely granular ground work. These two latter solutions are strong in the amount of acetic acid. What is perhaps the best fixative for preserving the corpuscles and filaments consists of chrom-acetic to which formalin has been added.

This fixative was recommended to the writer by M. l'Abbé Licent who used it with success in the botanical laboratory of the Sorbonne for fixing fungous tissues. His formula is:

A.	Chromic acid (2 percent)	80 parts
	Glacial acetic acid	5 parts
B.	Pure formalin (40 percent)	15 parts

A and B are mixed only at the time of fixing the material, and a change soon takes place in the fluid reducing the acetic acid and producing green compounds of chromium. The material may be left in the fluid for a few days, which then serves as a chromium mordant.

In general, it appears that the corpuscles and filaments here described are well preserved with osmium fixatives, even though some acetic acid is present. With strong acetic acid fixatives they are not preserved. Chromium compounds give good fixation in the presence of formalin. Once fixed, they are stained equally well with safranin or haematoxylin stains, though they stand out more clearly with the latter. Delafield's, Heidenhain's, and Weigert's haematoxylin methods were used with equal success.

In the old cytoplasm that is growing vacuolar, the granules are larger than in the younger, actively growing regions. Where the fruit bodies are attacked by the parasitic Tremella, the basidia undergo a sort of degeneration and present very large granules. Two such basidia are shown in the photomicrograph (fig. 16), and the basidium shown in figure 26 is drawn from parasitized tissue. If sections are cut of such parasitized plants in formalin (weak solution) with the freezing microtome and mounted in water, the large granules stain the characteristic red with Sudan III, showing that they are of a fatty nature and that in the course of degeneration of the cell the granules undergo a fatty metamorphosis.

In presenting this paper, the writer is more interested in reporting as accurately as possible the observations concerning the cytoplasmic structures in question than in urging any particular interpretation of them. There has been much written about mitochondria in the last decade. Yet we can hardly say that we know what they are. There is no specific technique that brings them out and delimits them from other cell constituents. Of the numerous functions attributed to them much is conjectured and little known for certain. It is difficult to believe that all the structures described by various writers in the cells of animal, fungous, and higher plant tissues are in the same category. The term *mitochondrium* therefore requires to be defined. If every granular or filamentous structure that appears in living cytoplasm and in cytoplasm fixed by various methods is to be called a mitochondrium, then the structures we here describe and figure are mitochondria. In that sense, it would be a generic term under which would fall various types of cytoplasmic structures. If, however, a mitochondrium is defined as a living organ of the cell with a specific function, with an individuality of its own, and with specific staining affinities, then these structures are not mitochondria. Kingsbury (14), in calling attention to the danger of basing morphological generalizations upon special technique without first ascertaining upon what the technique depends, speaks of the difference between these two views as "the issue . . . between a process interpretation of structure as against an elementary particle or material interpretation" (p. 47), and makes out a strong case in favor of the former.

In the cells of the radicle of Pisum the writer has obtained with various so-called mitochondrial fixatives the characteristic granular and rod-like structures commonly described as mitochondria. These can not be said to be identical with the similar structures described above. With Regaud's fixative the former are well brought out, while the latter are only poorly preserved. Flemming's medium solution and the formol-chrom-acetic fixative, on the other hand, fix the former structures (in Pisum) only poorly but preserve the latter well. The structures in the two kinds of tissue have this in common, however, that they are preserved by osmic and chromic fixatives and not with solutions too strong in acetic acid.

In 1902 Maire (17) described fibrils of kinoplasm in the basidia of certain species of Basidiomycetes studied by him, which extend from the daughter nuclei into the sterigmata and are thought to be concerned with the passage of the nuclei into the sterigmata. Fries (9) also makes bare mention of such structures in the basidia of Nidularia in the sentence (p. 155): "Bisweilen sind auch dünne Cytoplasmastränge vorhanden, die von den Kernen aus in die Sterigmaausbuchtungen hinein verliefen." Levine (15) describes and figures these kinoplasmic fibrils in certain species of the Boleti and believes he has determined that they are maintained as a continuous line of connection between the daughter nuclei of the basidium and the centrosomes from the time the former move downward into the basidium and the latter upward into the sterigmata and finally into the young spores themselves. Of their function he says (p. 173): "It is, perhaps, not entirely proven that these fibrillar strands are actively contractile kinoplasmic elements which pull the nuclei into the spores, but the appearances in the Boleti certainly suggest such a conclusion." The same author further says (p. 159): "At this stage I have also found another type of fibrils in the cytoplasm. These latter run irregularly but in the main lengthwise of the basidium. It may be that they are indications of cytoplasmic streaming." It seems likely that these latter fibrils are of the same type as those which I have observed in Aleurodiscus. As for kinoplasmic fibrils connecting the daughter nuclei of the basidium with the centrosomes in the sterigmata and spores, I have never seen them in Aleurodiscus. In the first place I can not establish the presence of centrosomes. In preparations fixed with the osmium and chromium fixatives it would be hopeless to try to find centrosomes among the numerous cytoplasmic granules unless they had well-defined astral rays, and in Carnoy and Zenker preparations no suggestion of centrosomes appears. At first sight, under low magnification, one can easily be deceived into thinking that the filiform structures one sees lead from the nuclei to the sterigmata, since so many of them are found in that region of the basidium. Higher magnifications easily clear up any such error. It might, however, be a timely warning to say that students working with species in which the basidia are small must be on guard against interpreting such structures as are here described as kinoplasmic fibrils connected with the daughter nuclei.

It is a significant fact that Juel (13), who used chrom-platinum-acetic and zinc chloride-acetic-alcohol fixatives in the study of the basidia of nineteen species of the genera Clavaria, Craterellus, and Cantharellus, figures in practically all cases a cytoplasm free from granules, fibrils, or similar structures. Of *Craterellus pistillaris* he says, however (p. 20): "Man sieht oft im Basidienplasma dichtere Plasmastränge, die von jedem Kern gegen die Basidienspitze hinziehen. Fädige Differenzierungen konnte ich in diesen Strängen nicht entdecken."

The writer believes that the granular and rod-like structures bear a relation to the reticular apparatus of Golgi. This characteristic structure, first reported in nerve cells, has since been reported by Golgi as well as other investigators to be present in a variety of different cells. Bensley (1) describes a similar structure in plant cells, and contends that with one type of fixation the vacuolar spaces are shown as separate isolated vacuoles while with a special Golgi technique they appear as a closed net. From the preparations of the writer, it is of course impossible to say in what condition the substance of the cytoplasmic structures in question exists in the living cells of Aleurodiscus. All fixation tends to produce in greater or lesser degree artificial conditions in the cell and it is necessary to interpret these artifacts in order to get at the nature of the substances in question in the living state. From the varying forms that it assumes with different types of fixation and from the fact that with certain fixations it disappears altogether, it seems reasonable for us to conclude that in the living protoplasm the substance exists in the form of a fluid with various substances in solution which have a nourishing function and among which are lipoids in colloidal solution. Where active growth takes place, as in rapidly enlarging basidia or growing sterigmata, the building of new protoplasm makes great demand on these substances and they are therefore present in abundance. They are attracted to points where active growth is taking place, and such attraction may be so strong as to set up definite lines of flowage which, when subjected to a fixative, coagulate in the form of the characteristic rods we have described. Such a manner of thinking of these structures explains their lying lengthwise of the hyphae and their direction towards points of growth. It fails, however, to explain why they should exist in degenerating basidia under the parasitic Tremella (see figs. 16 and 26).

In 1913, Löwschin (16) showed the numerous points of similarity between chondriosomes and myelin forms produced out of lecithin in different salt and albumin solutions under the conditions that lead to the formation of lecithinalbumins. Of the filaments observed, he says (p. 204): "Die langen Fäden, Spermatozoidformen und dergleichen bilden sich, wenn in der umgebenen Flüssigkeit Ströme existieren; die Ursache dieser Ströme mag verschieden sein."

Dangeard (6), in studying both living and fixed cells of Saprolegnia as well as of other plants, finds a canalicular system throughout the cytoplasm which he considers a nourishing apparatus, containing in colloidal solution of greater or lesser density substances of which the chemical nature has not yet been determined, but doubtless related to lipoids. They have the general character of blackening with osmic acid, and possess the same osmotic and elective properties as the metachromatin. They are precipitated in the form of solid granular or filamentous bodies under the influence of absolute alcohol and certain other reagents.

Speaking only for fungous tissue, we may conclude that many of the structures that have been described in the cytoplasm as morphological structures, mitochondria, metachromatic bodies, extranuclear chromatin, and the like, fall into a category of coagulation products resulting from a complex and variable fluid substance in the protoplasm that has the property of reducing osmium tetroxide and chromium salts and that is coagulable into definite structures. The tendency has been in general to interpret these structures from the morphological rather than from the physiological viewpoint.

SUMMARY

1. *Aleurodiscus amorphus* was collected on twigs and small branches of fallen balsam firs in the Adirondacks. Because of the large cells in the hymenium, it proved to be excellent material for cytological study. It is often parasitized by a Tremella which may completely cover and conceal the fruit bodies of the host. This is compared with other Tremellas that are associated with the fruit bodies of other fungi.

2. The mycelium of the Aleurodiscus grows throughout the intercellular spaces of the bark parenchyma of the twigs and small branches. The fruit body begins its development as a mass of densely interwoven hyphae within the lower tissues of the bark. There is an upward growth of the hyphae, and they emerge through the ruptured corky and epidermal layers. Here they branch and expand into the characteristic fruit body that is typically convex but may be more or less pezizoid in form. The hymenium is characterized by the nodulose paraphyses.

3. In the cytoplasm fixed with osmium and chromium fixatives, large filaments and numerous granules appear, which are thought to be in a class with mitochondria, metachromatic bodies, and other morphological struc-

tures that have been described in the cytoplasm of the fungi. They are interpreted as coagulation products of a fluid vacuolar sap that has lipoid substances in solution. In degeneration under the parasitic Tremella they undergo a fatty metamorphosis.

The writer wishes to make grateful acknowledgment to Professor B. F. Kingsbury for the help he has given in the study of cytoplasmic granules in general and to Professors W. W. Rowlee and H. M. Fitzpatrick for their helpful suggestions in the preparation of the manuscript.

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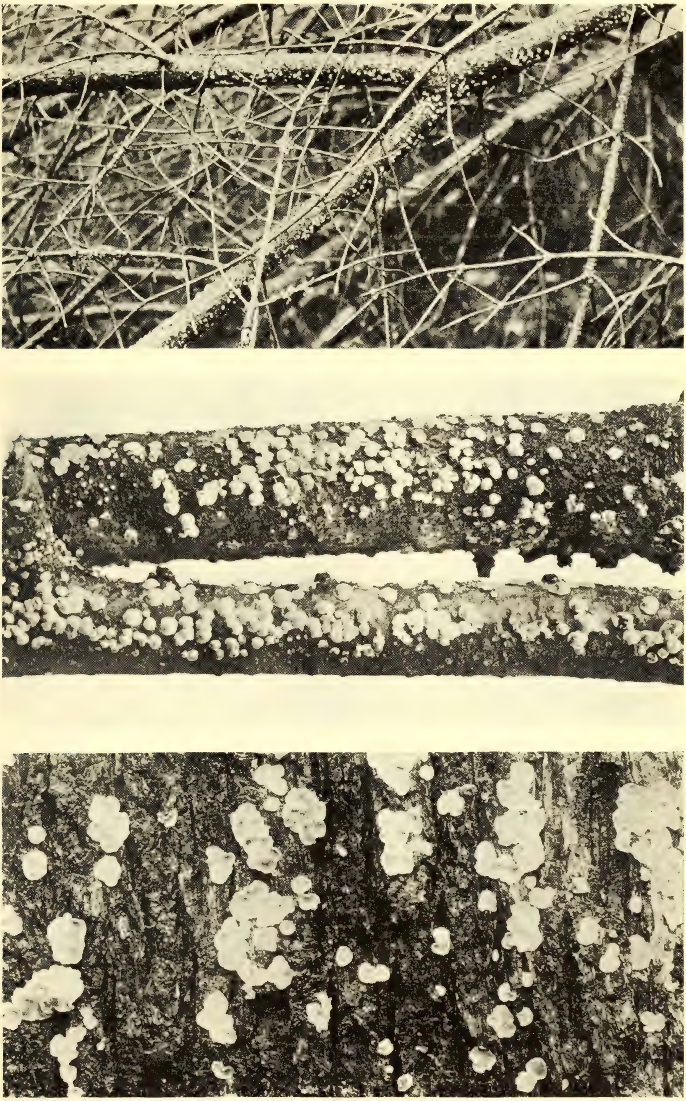
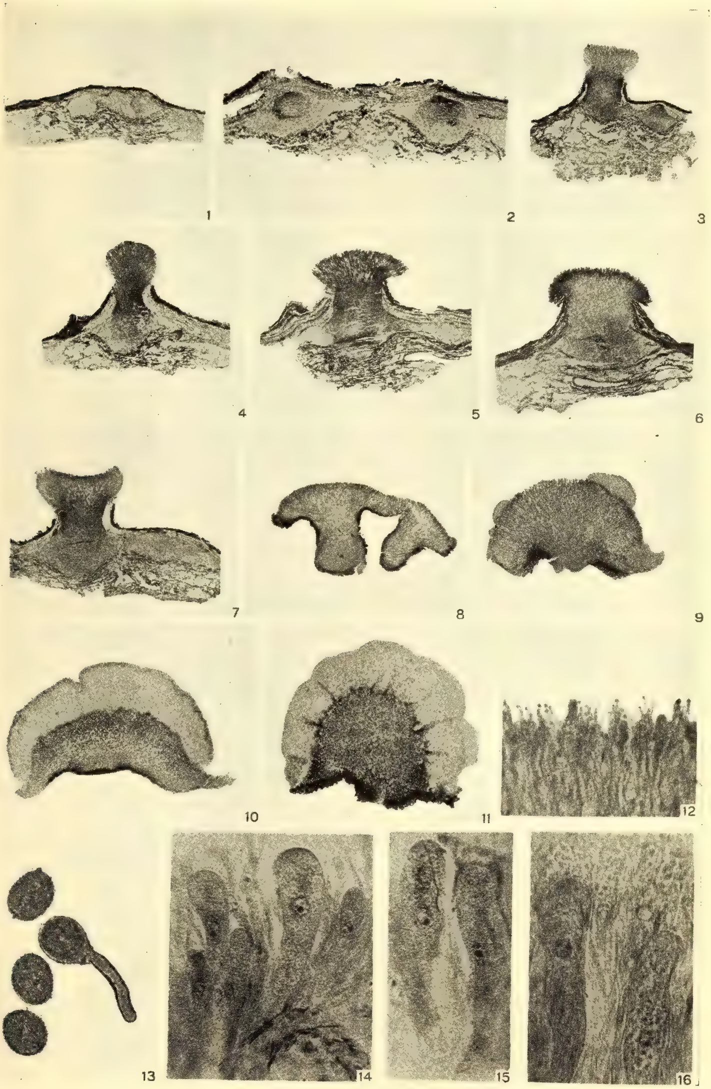


Stork: Biology and structure of Aleurodiscus.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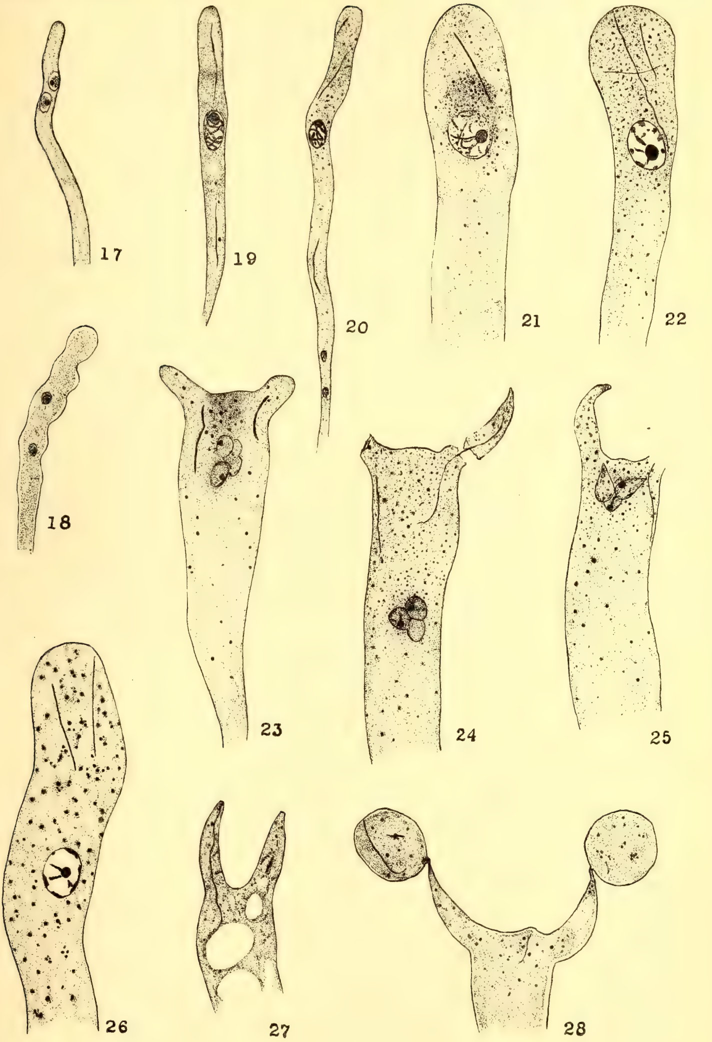
STORK: BIOLOGY AND STRUCTURE OF ALEURODISCUS.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STORK: BIOLOGY AND STRUCTURE OF ALEURODISCUS.

EXPLANATION OF PLATES

PLATE XXXI

Upper figure: *Aleurodiscus amorphus* on Abies twigs. ¼ natural size.
Middle: Same, natural size.
Lower: *Aleurodiscus Oakesii* on bark of living Ostrya. Natural size.

PLATE XXXII

Figures 1–11, × 12. Figure 12, × 84. Figure 13, × 440. Figures 14–16, × 390.

FIG. 1. Section of bark of Abies with a stroma of pseudoparenchymatous tissue of *Aleurodiscus amorphus*, the primordium of a fruit body.

FIG. 2. Two similar primordia somewhat further advanced.

FIG. 3. A fruit body that has broken through the outer layers of bark and a smaller one about to emerge by its side.

FIGS. 4–6. Further development of the same. In the fruit body shown in figure 6 the hymenium has begun to develop.

FIG. 7. Pezizoid type of fruit body. The numerous thin white hyphae on its sides and margin give it a tomentose appearance.

FIG. 8. Two confluent fruit bodies.

FIG. 9. Fruit body with three stromata of *Tremella* sp. growing upon it.

FIG. 10. Later stage of a similar fruit body, the Tremella having entirely covered the host. The hymenial layer of globose basidia appears as a border of black dots on the upper surface.

FIG. 11. Similar to preceding.

FIG. 12. Section of hymenium of *Aleurodiscus amorphus* showing basidia and paraphyses. One older basidium is seen with two sterigmata in the section.

FIG. 13. Germination of basidiospore.

FIG. 14. Basidia and paraphyses. Flemming fixation. The granules are evident in the basidia, but no filaments lie in the plane of focus.

FIG. 15. Basidia. Formol-chrom-acetic fixation. Granules and one filament visible.

FIG. 16. Two basidia in a hymenium, above which is seen the tissue of the parasitic Tremella. Notice the larger corpuscles in the basidia.

PLATE XXXIII

(All figures × 880)

FIG. 17. Filiform paraphysis.

FIG. 18. Nodulose paraphysis.

FIG. 19. Young basidium showing granules and filaments.

FIG. 20. Basidium in lower part of hymenium, perhaps old but crowded for room and unable to elongate. No cross wall is here present.

FIG. 21. Growing basidium showing cap of granules and one filament above the fusion nucleus.

FIG. 22. Several filaments visible, one occupying an exceptional horizontal position.

FIG. 23. Aggregation of granules apparently proceeding to apex of basidium. Filaments tending toward the growing sterigmata.

FIG. 24. Sterigma broken loose from the basidium in the course of preparation, the filament remaining intact.

FIG. 25. Beaked daughter nuclei ready to proceed into sterigmata. No fibrils are seen attached to them.

FIG. 26. Degenerating basidium from hymenium parasitized by *Tremella* sp. The large corpuscles stain with Sudan III.

FIG. 27. Old vacuolate basidium after sporulation.

FIG. 28. Spores in process of formation. The one at the left shows a filament and what is probably a nuclear spindle.

THE GERMINATION OF THE SPORES OF CONOCEPHALUM CONICUM

Sister M. Ellen

Introduction

This work was undertaken in order to determine the time and the extent of the germination of the spores within the capsule of *Conocephalum conicum* (L.) Dumort.

In 1895 Farmer (3) stated that the spores of this species germinate before leaving the capsule, but that the divisions of the spore do not occur so early as in Pellia, in which plant germination sometimes occurs even before the separation of the four spores of a tetrad.

In 1903 Cavers (2) described the "mature spore" of Conocephalum as an ovoid mass of five or six cells.

In 1905 Bolleter (1) reported that the divisions of the spores begin in the spring and that the "mature spore" is many-celled.

Material and Methods

The plants used for these investigations were collected in the fall and spring of the years 1918–19 and 1919–20 on a bluff in almost the extreme southwestern corner of the state of Wisconsin about three miles southwest of Sinsinawa Mound.

The material used for the study of the spores and of their germination under normal conditions was collected beginning September 1, 1918, and continuing at intervals of not more than two weeks until December 20. The collections were resumed on March 7, 1919, and continued until April 17, at which time spore dispersal had already begun. The sporophytes were put into the killing fluid either in the field or immediately after being brought into the laboratory.

The most successful of the killing fluids used were Flemming's medium solution for the fall and early spring stages, and Flemming's strong solution for the later spring stages. The material was imbedded in 53° paraffin and microtome sections were made from seven to nine microns in thickness. All stages were studied from the fresh material as well as in prepared sections.

Immediately after dispersal, some of the sporelings[1] were sown in rain water in petri dishes and placed, some in a north exposure and others in a south exposure. Sporelings which were kept in a paper packet for thirty-six days after dispersal were sown on tap water in order to study the effect of drying on their power of further development.

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[1] The multicellular structures resulting from the intra-capsular germination of the spores have been commonly referred to by previous writers as "spores" or "mature spores." They are, of course, strictly speaking, young gametophytes, and will be spoken of in the present paper as sporelings.

In order to observe the effect of artificial conditions on the sporelings, gametophytes bearing carpocephala were collected in the latter part of October and on November 28, 1919. A spring collection was made on March 17, 1920, when the plants were still frozen. All the material when collected showed five, six, or seven cells in the sporelings, and was, so far as could be determined, in about the same condition as the material which was collected in October and November, 1918, and on March 7, 1919. The plants were placed under bell jars in a north room the temperature of which seldom exceeds 68° F. From time to time the plants were watered according as they showed signs of drying.

Germination and Development under Normal Conditions

The spore mother cell is, as described by Farmer (3), a large, flattened, oval body rather the shape of a biscuit. The nucleus is large and, in the material studied, appeared as a homogeneous mass (Pl. XXXIV, fig. 1), showing that the spore mother cells were evidently in that stage previous to tetrad formation in which Farmer found the nucleus to take the stains in a similar manner.

The spores, still united in tetrads, were examined from the living material on September 15 and were easily discerned as three-faced pyramids enclosed within a thin mother cell wall. The stained preparations of this material were almost altogether unsatisfactory because of contraction in the cells (fig. 2). Farmer also reports this stage as being particularly difficult to fix satisfactorily.

As early as September 21, the spores were already freed from the mother cell wall although they were not yet fully rounded. Gradually they took on a somewhat globular form and the spore wall was differentiated into two layers, the outer one of which was golden brown in color and much dotted with tiny, bead-like projections. At the same time a large amount of starch was laid down in the spores and there was an increase in the size of the nucleus and of the spore itself (fig. 3).

Cell division in the spores was found as early as October 5, but this process did not go on simultaneously in every capsule of a single head, nor, indeed, in every spore within a given capsule. Some capsules were found wherein no divisions had occurred and others in which there were one-, two-, and four-celled sporelings (figs. 3, 4, 5).

According to Bolleter (1) the spore of *Concephalum conicum* remains in the one-celled stage throughout the winter. Possibly climatic conditions are the cause of the difference between his results and mine, since the material studied by him was collected near Zürich. But since he records no collection later than the beginning of October, and none thereafter earlier than the return of growing weather in March, the question arises as to whether the first divisions, which had already taken place in the fall, might have been thought by him to be spring divisions. The fact that the times stated for tetrad formation, stalk elongation, spore dispersal, and other activities of the plant, as observed by Bolleter, seem to be approximately the same as in material studied by me, makes it seem still more likely than his statement regarding the time of the first divisions of the spores is erroneous.

The partition walls when first formed between the cells of a sporeling are notably thin, and with Flemming's triple stain are scarcely discerniible Light green, when used instead of Orange G, however, gave them sufficient prominence to make them easily visible. After the first three divisons. have occurred (fig. 6), there is a slight enlargement of the cells, a thickening of the partition walls, and a deposition of starch (fig. 7).

Material collected on March 7 showed no signs of change as compared with the late fall and winter collections, but on the 19th of March, the first really spring-like day, further divisions within the spore wall had taken place. Division stages were also observed in some of the preparations from this material. Within a few days the sporelings showed as many as ten or eleven cells in a median longitudinal section (fig. 8). Again there was a stoppage of cell division, and a period followed during which time there was growth and rapid development of chlorophyll within the cells. The next series of cell divisions took place early in April, and by April 9 the sporelings showed as many as seventeen cells in a median longitudinal section (fig. 9). It was noted that each period of cell division in the sporelings was preceded by growth and by the production and storage of starch.

During the latter part of this series of divisions, but more especially after the process was completed, the stalks of the carpocephala elongated rapidly until the sessile heads were raised five or six centimeters above the thallus. This rapid elongation is due, as is stated by Cavers (2) and Bolleter (1), to the growth of the cells already formed rather than to cell divisions within the stalks.

The sporelings which were sown immediately after dispersal almost without exception promptly resumed their development, and within less than twenty-four hours the majority showed at least two, and many of them showed three rhizoids (figs. 10, 11). A bud destined to develop into a thallus (*b*, fig. 12), usually appeared within four or five days, and a little later, secondary rhizoids were developed from the growing thallus (fig. 13). The above description holds for those sporelings which were placed in weak, as well as for those placed in strong, illumination. But in the later stages there was a relatively rapid and profuse development of both thalli and secondary rhizoids in the material which was placed in strong illumination (Pl. XXXV, figs. 14, 15).

To test the effect of drying, some of the sporelings which had been kept in a paper packet for thirty-six days were sown on tap water and placed in moderate light. For a few days there seemed to be no development, though there was a significant distention and a marked suggestion of chlorophyll development in individual sporelings. No certain evidence of the development of thalli was observed in this culture for several days, but within a week or so, probably as many as ten percent of the sown sporelings began to develop further. However, the greater number of those sporelings which developed thalli formed no primary rhizoids (figs. 16, 17). This would seem to indicate that, as Cavers (2) and Bolleter (1) have reported, the cells which normally develop rhizoids are more susceptible to desiccation than are the other cells of the sporelings. A very small percentage of these dried sporelings, nevertheless, developed in an apparently perfectly normal way.

Effect of Artificial Conditions

In the material collected in the fall, very little change was noted as late as December 15, when some of the sporelings of the collection made in November were sown on water. Sporelings from plants collected in late October, after being indoors nearly three months, were sown, but only a very small number of these showed a history of growth, cell division, and the development of rhizoids similar to that described below.

Of the sporelings collected in November, the majority developed after being sown. Many showed one primary rhizoid each, very few showed two, and some showed none (figs. 18, 19). The young thalli developed rapidly and all sent out secondary rhizoids, although the latter were proportionately few in number (figs. 20, 21) as compared with those of the thalli which developed from sporelings collected and sown in the spring (figs. 11, 15).

Toward the end of December, the stalks of the carpocephala of the plants collected in November began to elongate, though slowly, until they were about two centimeters in length. There was also a corresponding lengthening of the setae of the sporophytes. During a period of ten days or more, there was no further advance toward spore dispersal. The capsules ruptured the calyptra and the enveloping sheath, but the setae did not elongate sufficiently to permit the ordinary dehiscence and sporeling dissemination. Some of the sporelings from these capsules were sown, however, and within less than thirty-six hours a few showed signs of development, and in time practically all of them developed as shown in figures 22–25.

The plants collected on March 17 showed signs of the elongation of the stalks of the carpocephala on the second day after being brought into the laboratory. The old gametophytes developed each a new thallus by means of growth from the apical region. After these plants had been in the laboratory for a week, the stalks had grown still further, until on April 2 some of them were as much as six centimeters in length. This was accompanied by the lengthening of the setae of the sporophytes, the rupturing of the calyptras, growth of the enveloping sheaths, the dehiscence of the capsules, and finally by an apparently normal dispersal of the sporelings.

An examination of these sporelings from time to time, previous to and following the elongation of the stalks of the carpocephala, showed no change in them until the stalks were about three centimeters in length. Then there occurred a slight increase in size of the sporelings, cell divisions, and good chlorophyll development. The sporelings apparently did not develop any further before their dispersal unless it was that there was more extensive chlorophyll development than before the divisions. At the time of dispersal the sporelings showed only 8 to 12 cells each, instead of 30 to 40 cells as is the case with the sporelings dispersed under natural out-of-door conditions. All these sporelings were still enclosed within the spore walls. Some of them were sown, and their subsequent development was almost as rapid as had been observed in those that were subjected to normal out-of-door conditions. The number of primary rhizoids, however, was never more than two, and in most cases there was but one to each sporeling (figs. 26, 28). The number of secondary rhizoids in each case was also small as compared with the number produced by thalli which grew from normally developed sporelings.

Cavers' (2) description of the mature "spores" of *Conocephalum conicum*, both as to the number of their cells and the number of primary rhizoids developed subsequent to their being sown, is very similar to my observations just detailed on plants which had been subjected to artificial conditions. It seems possible, therefore, that the plants which he studied were also subjected to other than perfectly natural conditions.

Summary

1. The spore mother cells of *Conocephalum conicum* are well developed in this region before September first, and the spores are freed from the spore mother cell walls about the middle of September. Toward the end of the month the spores are well-rounded, rough-walled, and each contains a relatively large nucleus.

2. In the early part of October cell division begins, this being preceded by growth and by a heavy deposit of starch. Before the middle of the month as many as five cells are seen in a median longitudinal section, so that there are probably as many as six to eight cells in each sporeling. These cells all remain within the spore wall.

3. Before winter sets in, there is a thickening of the partition walls, a deposition of starch, and growth in the cells formed by the division of the spores. In this condition the usually six- to eight-celled sporelings rest through the winter.

4. Cell division is resumed with the coming of the first warm weather in the spring and proceeds rapidly until the stored food is consumed. Then, during a pause in cell division, there is growth of the cells, a rapid

development of chlorophyll and of starch, followed by a second series of cell divisions until each sporeling has developed into a nearly spherical mass of from thirty to forty cells.

5. A short time before the cell divisions are complete the stalks of the carpocephala begin to lengthen, and during four or five days after the sporelings have matured, these stalks elongate rapidly until they attain a height of five or six centimeters.

6. Simultaneous with the rapid elongation of the stalks is a lengthening of the setae of the sporophytes by means of which the capsule is thrust through the calyptra and the enveloping sheath. The capsule wall is then ruptured and the sporelings and elaters are dispersed.

7. The sporelings are on the whole rather short-lived, though some are capable of developing thalli after being dried for as long as thirty-six days.

8. The sporelings collected in the late fall develop subsequent to their being sown even though there are no stalk elongation, no spore dispersal, and no cell divisions previous to the sowing after those which occurred under natural conditions.

9. The stalks of the carpocephala, which normally begin elongation after a series of spring divisions in the cells of the sporelings, elongate before such divisions occur if the plants are brought indoors.

10. The number of cells in a naturally developed sporeling before dispersal is from thirty to forty, while the number of cells in the sporeling which has been subjected to artificial conditions is from five to twelve according as these conditions more or less closely approach the natural ones.

11. The number of primary rhizoids produced by a normal sporeling ranges from three to five, while the number from the sporeling placed under artificial conditions is never more than two, most often only one, and in some cases no primary rhizoid is produced. The number of secondary rhizoids from the thalli developed from sporelings subjected to artificial conditions is also relatively few as compared with the number developed from the thalli of naturally developed sporelings.

I wish to express my sincere thanks to Professor C. E. Allen who directed the writing of this paper, for his many helpful suggestions and criticisms, and to Dr. W. N. Steil who suggested the work, for his encouragement and assistance during its progress.

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EXPLANATION OF PLATES

All figures were made with the aid of a camera lucida and drawn at table level. Leitz objective no. 6 and ocular no. 4 were used in making figures 1–9; objective no. 3 and ocular no. 4 were used in drawing figures 10–17. With a tube length of 170 mm., a magnification of about 660 was obtained in figures 1–9 and a magnification of 140 in figures 10–17. For figures 18–28 a Bausch & Lomb 16 mm. objective and 12.5 mm. ocular gave a magnification of 200. These magnifications were reduced by one-third in reproduction.

The following abbreviations are used: *r*, rhizoid; *b*, bud; *s*, sporeling; *y t*, young thallus; *s c*, secondary rhizoid.

PLATE XXXIV

FIG. 1. A spore mother cell.

FIG. 2. Spore tetrad within the mother cell wall.

FIG. 3. Spore just before its first division.

FIG. 4. Two cells formed by the division of a spore and remaining surrounded by the spore wall.

FIG. 5. Four cells formed within the spore wall.

FIG. 6. Median longitudinal section of a sporeling showing five cells. Probably as many as eight cells are present.

FIG. 7. Same as figure 6, after the thickening of the walls and the deposition of starch.

FIG. 8. Condition of sporeling during the first series of spring divisions.

FIG. 9. Median longitudinal section of a sporeling ready for dispersal.

FIG. 10. First stage in external development. Sporeling twelve hours after being sown on rain water.

FIG. 11. Sporeling about twenty-four hours after being sown on rain water.

FIG. 12. Later stage showing the development of a bud (*b*) which is to grow into a thallus.

FIG. 13. Gametophytes about six days old showing secondary rhizoids.

PLATE XXXV

FIGS. 14, 15. Gametophytes developed in strong light.

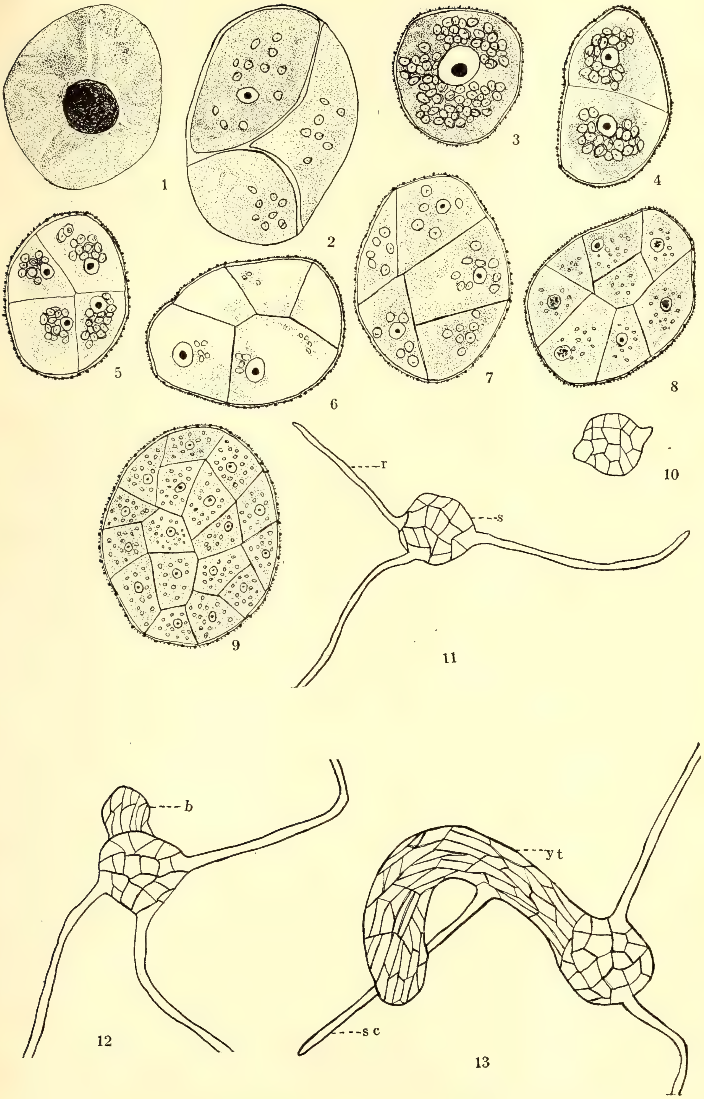
FIGS. 16, 17. Sporeling which had suffered desiccation before being sown. No rhizoids have developed.

FIGS. 18, 19. Developing sporelings which were removed from the capsule before there was any advance toward spore dispersal.

FIGS. 20, 21. Later stages in the development of gametophytes treated like those shown in figures 18 and 19.

FIGS. 22–25. Stages in the external development of sporelings from the same collection as those shown in figures 18 to 21, but which were not sown until after there was some elongation of the stalks of the carpocephala and of the setae of the sporophytes.

FIGS. 26–28. Stages in the external development of sporelings subjected to indoor conditions beginning March 7, and after which they were dispersed naturally.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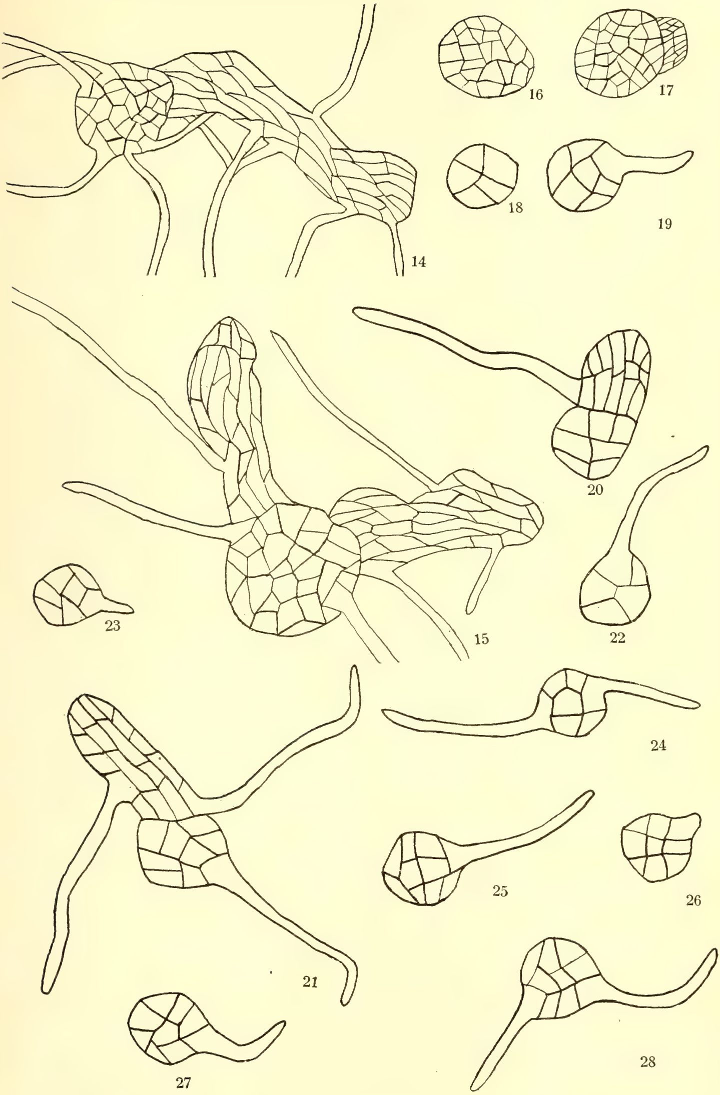
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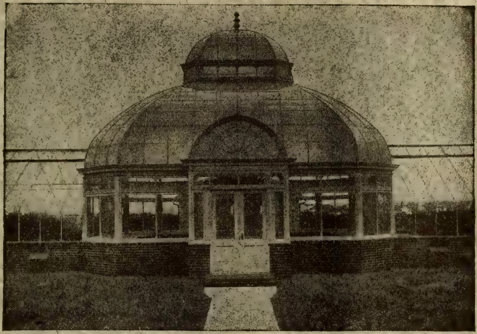

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